



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :  A61K 39/385, A01N 37/00, 43/02, 31/14, C08F 283/00		A1	(11) International Publication Number: WO 99/53951  (43) International Publication Date: 28 October 1999 (28.10.99)
(21) International Application Number: PCT/US99/08373			(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
(22) International Filing Date: 16 April 1999 (16.04.99)			
(30) Priority Data: 09/062,305 17 April 1998 (17.04.98) US			
(71) Applicant: ENZON, INC. [US/US]; 20 Kingsbridge Road, Piscataway, NJ 08854 (US).			
(72) Inventors: MARTINEZ, Anthony, J.; 20 Weybourne Road, Hamilton Square, NJ 08690 (US). PENDRI, Annapuma; 1221 Town Colony Drive, Middletown, CT 06457 (US). GREENWALD, Richard, B.; 113 Hickory Road, Somerset, NJ 08873 (US). CHOE, Yun, H.; 2 Gemma Court, Piscataway, NJ 08854 (US).			
(74) Agents: MERCANTI, Michael, N. et al.; Roberts & Mercanti, L.L.P., P.O. Box 484, Princeton, NJ 08542-0484 (US).			
(54) Title: TERMINALLY-BRANCHED POLYMERIC LINKERS AND POLYMERIC CONJUGATES CONTAINING THE SAME			
<p>Structure (1) shows a general formula where E<sub>1</sub> is attached to a nitrogen atom, which is further attached to a carbon atom. This carbon atom is bonded to two groups: Y<sub>1</sub> and R<sub>2</sub>, and to another carbon atom. The second carbon atom is bonded to M and R<sub>1</sub>. Structure (2) shows a branched linker where B is attached to a central carbon atom, which is bonded to two Y<sub>1</sub> groups and two R<sub>2</sub> groups. This central carbon atom is also bonded to two other carbon atoms, each of which is bonded to R<sub>3</sub> and R<sub>4</sub> groups. Structure (3) is similar to (2), but the two side chains are labeled (m1) and (m2). Structure (4) shows a linker where E<sub>1</sub> is attached to a nitrogen atom, which is further attached to a carbon atom. This carbon atom is bonded to two R<sub>2</sub> groups and to another carbon atom. Structure (5) shows a linker where M is attached to a carbon atom, which is bonded to two R<sub>2</sub> groups and to another carbon atom. This final carbon atom is bonded to two E<sub>4</sub> groups and to a nitrogen atom.</p>			
(57) Abstract			
<p>The present invention is directed to polymeric prodrug transport forms of formula (I), wherein: J is (1), (2) or (3), E<sub>1-4</sub> are independently selected from the group consisting of hydrogen, C<sub>1-6</sub> alkyls, C<sub>3-12</sub> branched alkyls, C<sub>3-8</sub> cycloalkyls, C<sub>1-6</sub> substituted alkyls, C<sub>3-8</sub> substituted cycloalkyls, aryls, substituted aryls, aralkyls, C<sub>1-6</sub> heteroalkyls, substituted C<sub>1-6</sub> heteroalkyls, C<sub>1-6</sub> alkoxy, phenoxy, C<sub>1-6</sub> heteroalkoxy, (2) or (3) and at least one of E<sub>1-4</sub> includes a B moiety, wherein B is a leaving group, OH, a residue of a hydroxyl- or amino-containing moiety or formula (II) wherein J<sub>1</sub> is the same as J, or another member of the group defining J and E<sub>5</sub> is the same as E<sub>1-4</sub>, or another member of the group defining E<sub>1-4</sub>; Y<sub>1-2</sub> are independently O, S or NR<sub>9</sub>; M is a heteroatom selected from either X or Q; wherein X is an electron withdrawing group and Q is a moiety containing a free electron pair positioned three to six atoms from C(=Y<sub>2</sub>); R<sub>2-5</sub> and R<sub>7-9</sub> are independently selected from the group consisting of hydrogen, C<sub>1-6</sub> alkyls, C<sub>3-12</sub> branched alkyls, C<sub>3-8</sub> cycloalkyls, C<sub>1-6</sub> substituted alkyls, C<sub>3-8</sub> substituted cycloalkyls, aryls, substituted aryls, aralkyls, C<sub>1-6</sub> heteroalkyls, substituted C<sub>1-6</sub> heteroalkyls, C<sub>1-6</sub> alkoxy, phenoxy and C<sub>1-6</sub> heteroalkoxy; (m1) and (m2) are independently zero or one; (n1), (n2), (p1), (p2) and (q) are independently zero or a positive integer; Z is an electron withdrawing group; and R<sub>1</sub> is a polymeric residue, which is optionally capped with a moiety of formula (V).</p>			

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

## TERMINALLY-BRANCHED POLYMERIC LINKERS AND POLYMERIC CONJUGATES CONTAINING THE SAME

### TECHNICAL FIELD

The present invention relates to new types of terminally-activated polymeric materials which are useful in forming long-acting conjugates of bioactive materials. In particular, the invention relates to polymeric-based conjugates having increased therapeutic payloads and methods of preparing the same.

### BACKGROUND OF THE INVENTION

Over the years, several methods of administering biologically-effective materials to mammals have been proposed. Many medicinal agents are available as water-soluble salts and can be included in pharmaceutical formulations relatively easily. Problems arise when the desired medicinal agent is either insoluble in aqueous fluids or is rapidly degraded *in vivo*. Alkaloids are often especially difficult to solubilize.

One way to solubilize medicinal agents is to include them as part of a soluble prodrug. Prodrugs include chemical derivatives of a biologically-active parent compound which, upon administration, eventually liberate the parent compound *in vivo*. Prodrugs allow the artisan to modify the onset and/or duration of action of an agent *in vivo* and can modify the transportation, distribution or solubility of a drug in the body. Furthermore, prodrug formulations often reduce the toxicity and/or otherwise overcome difficulties encountered when administering pharmaceutical preparations. Typical examples of prodrugs include organic phosphates or esters of alcohols or thioalcohols. See Remington's Pharmaceutical Sciences, 16th Ed., A. Osol, Ed. (1980), the disclosure of which is incorporated by reference herein.

5 Prodrugs are often biologically inert or substantially inactive forms of the parent or active compound. The rate of release of the active drug, i.e. the rate of hydrolysis, is influenced by several factors but especially by the type of bond joining the parent drug to the modifier. Care must be taken to avoid preparing prodrugs which are eliminated through the kidney or reticular endothelial system, etc. before a sufficient amount of hydrolysis of the parent compound occurs.

10 Incorporating a polymer as part of a prodrug system has been suggested to increase the circulating life of a drug. However, it has been determined that when only one or two polymers of less than about 10,000 daltons each are conjugated to certain biologically active substances such as alkaloid compounds, the resulting conjugates are rapidly eliminated *in vivo*, especially if a somewhat hydrolysis-resistant linkage is used. In fact, such conjugates are so rapidly cleared from the body that even if a hydrolysis-prone ester linkage is used, not enough of the parent molecule is regenerated *in vivo* to be therapeutic.

15 Camptothecin and related biologically active analogs are often poorly water soluble and are examples of substances which would benefit from PEG prodrug technology. A brief overview of some previous work in the field is presented below.

20 Ohya, et al., J. Bioactive and Compatible Polymers Vol. 10 Jan., 1995, 51-66, disclose doxorubicin-PEG conjugates which are prepared by linking the two substituents via various linkages including esters. The molecular weight of the PEG used, however, is only about 5,000 at most. Thus, the *in vivo* benefits are not fully realized because the conjugates are substantially excreted prior to sufficient linkage hydrolysis.

25 U.S. Patent No. 4,943,579 discloses certain simple 20(S)-camptothecin amino acid esters in their salt forms as water soluble prodrugs. The reference does not, however, disclose using an amino acid as part of a linkage which would attach the alkaloid to a relatively high molecular weight polymer in order to form a prodrug. As evidenced by the data provided in Table 2 of the '579 patent, hydrolysis is rapid. Consequently, at physiologic pH, the insoluble base is rapidly generated after injection, binds to proteins and is quickly eliminated from the body

before a therapeutic effect can be achieved. A related effort was directed to developing a water-soluble camptothecin sodium salt. Unfortunately, the water-soluble sodium salt of camptothecin remained too toxic for clinical application (Gottlieb et al., 1970 *Cancer Chemother. Rep.* 54, 461; Moertel et al., 1972 *ibid.* 56, 95; Gottlieb et al., 1972 *ibid.* 56, 103).

5

Commonly-assigned PCT publication WO96/23794 describes bis-conjugates in which one equivalent of the hydroxyl-containing drug is attached to each terminal of the polymer. In spite of this advance, techniques which would further increase the payload of the polymer have been sought.

10

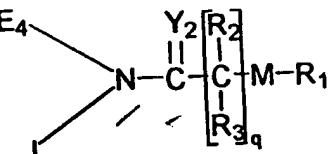
Thus, there continues to be a need to provide additional technologies for forming prodrugs of therapeutic moieties such as camptothecin and related analogs. The present invention addresses this need.

15.

### SUMMARY OF THE INVENTION

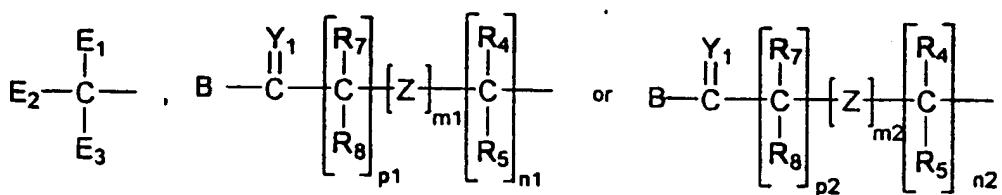
In one aspect of the invention, compounds of Formula (I) are provided:

(I)

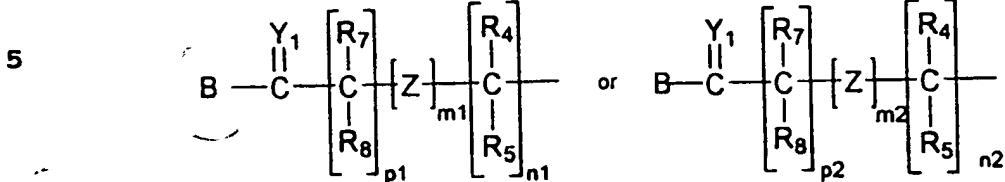


wherein:

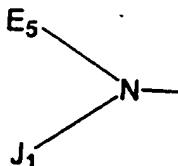
J is



$E_{1-4}$  are independently selected from the group consisting of hydrogen,  $C_{1-6}$  alkyls,  $C_{3-12}$  branched alkyls,  $C_{3-8}$  cycloalkyls,  $C_{1-6}$  substituted alkyls,  $C_{3-8}$  substituted cycloalkyls, aryls, substituted aryls, aralkyls,  $C_{1-6}$  heteroalkyls, substituted  $C_{1-6}$  heteroalkyls,  $C_{1-6}$  alkoxy, phenoxy,  $C_{1-6}$  heteroalkoxy,



and at least one of  $E_{1-4}$  includes a B moiety, wherein B is a leaving group, OH, a residue of a hydroxyl- or amine- containing moiety or



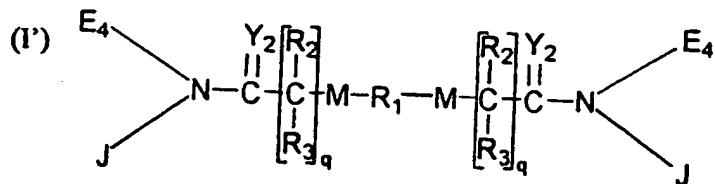
wherein  $J_1$  is the same as J, or another member of the group defining J and  $E_5$  is the same as  $E_{1-4}$ , or another member of the group defining  $E_{1-4}$ ;

10  $Y_{1-2}$  are independently O or S;  
M is a heteroatom selected from either X or Q; wherein X is an electron withdrawing group and Q is a moiety containing a free electron pair positioned three to six atoms from  $C(=Y_2)$ ;

15  $R_{2-3}$  and  $R_{7-8}$  are independently selected from the group consisting of hydrogen,  $C_{1-6}$  alkyls,  $C_{3-12}$  branched alkyls,  $C_{3-8}$  cycloalkyls,  $C_{1-6}$  substituted alkyls,  $C_{3-8}$  substituted cycloalkyls, aryls, substituted aryls, aralkyls,  $C_{1-6}$  heteroalkyls, substituted  $C_{1-6}$  heteroalkyls,  $C_{1-6}$  alkoxy, phenoxy and  $C_{1-6}$  heteroalkoxy;

(m1) and (m2) are independently zero or one;  
(n1), (n2), (p1), (p2) and (q) are independently zero or a positive integer;  
20 Z is an electron withdrawing group; and  
 $R_1$  is a polymeric residue such as a water-soluble polyalkylene oxide, preferably having a molecular weight of at least about 20,000 Daltons.

In preferred aspects of the invention, the polymeric residue is also substituted on the distal portion with another branching group to provide compounds of the formula (I'):



5 where all variables are as previously defined. The bifunctional compounds are thus formed when the polymeric residue ( $R_1$ ) includes both an alpha and an omega terminal linking group so that two, four or more equivalents of a biologically active agent, drug or protein, designated herein as B, can be delivered. Multifunctional compounds represented by the formula (I') are preferred.

10 When B is a residue of a hydroxyl-containing moiety or an amine-containing moiety, each B is attached via a hydrolyzable linkage which attaches to the polymer residue terminus.

15 Examples of hydroxyl-containing moieties for which one or more of improved aqueous solubility, decreased antigenicity, prodrug and/or controlled release delivery is desired include chemotherapeutic compound residues such as anti-fungal compounds, including triazoles, echinocandins, pneumocandins, etc, anti-cancer compounds such as camptothecin, paclitaxel, etoposide, anti-cancer platinum compounds containing OH groups, floxuridine or podophyllotoxin. In still further embodiments, other oncolytic agents, non-oncolytic agents such as anti-inflammatories, including steroidal compounds, as well as therapeutic low molecular weight peptides such as insulin are also contemplated.

20 Examples of amine-containing moieties for which one or more of improved aqueous solubility, decreased antigenicity, prodrug and/or controlled release delivery is desired include antimetabolites such as Ara-C or gemcitabine.

Alternatively, B can be a leaving group such as N-hydroxybenzotriazolyl, N-hydroxyphthalimidyl, halogen, *p*-nitrophenoxy, imidazolyl, N-hydroxysuccinimidyl, thiazolidyl thione, or other activating groups.

For purposes of the present invention, the term "residue" shall be  
5 understood to mean that portion of a biologically active compound which remains  
after the biologically active compound has undergone a substitution reaction in  
which the prodrug carrier portion has been attached.

10 For purposes of the present invention, the term "alkyl" shall be understood  
to include straight, branched, substituted, e.g. halo-, alkoxy-, and nitro- C<sub>1-12</sub> alkyls,

C<sub>3-8</sub> cycloalkyls or substituted cycloalkyls, etc.

15 For purposes of the present invention, the term "substituted" shall be  
understood to include adding or replacing one or more atoms contained within a  
functional group or compound with one or more different atoms.

20 The term "sufficient amounts" for purposes of the present invention shall  
mean an amount which achieves a therapeutic effect as such effect is understood by  
those of ordinary skill in the art.

25 One of the chief advantages of the compounds of the present invention is  
that the prodrugs have a higher payload per unit of polymer than previous  
techniques. Another advantage is that the linkers achieve a proper balance between  
the rate of parent drug-polymer linkage hydrolysis and the rate of clearance of  
prodrug from the body. The linkages between the polymer and the parent  
compounds, also referred to herein as a biologically-active nucleophiles, hydrolyze  
at a rate which allows a sufficient amount of the parent molecules to be released in  
vivo before clearance of the prodrug from the plasma or body. The high payload  
polymeric conjugates of the present invention are thus unique delivery systems  
which can contain up to four or a greater number of molecules of a drug.

Methods of making and using the compounds and conjugates described  
herein are also provided.

**BRIEF DESCRIPTION OF THE DRAWINGS**

Figures 1a, 1b, 2a, 2b schematically illustrate methods of forming compounds of the present invention.

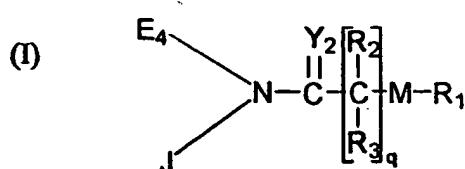
Figures 3-9 schematically illustrate compounds synthesized in the Examples.

5

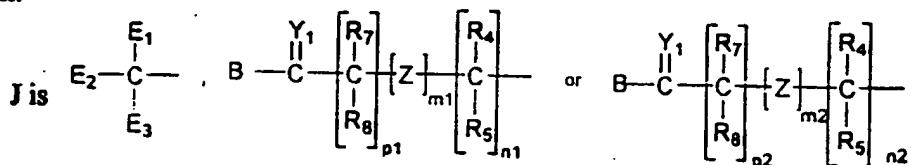
**DETAILED DESCRIPTION OF THE INVENTION****A. THE PRODRUGS**

In one preferred embodiment of the invention, the compositions of the invention comprise the formula:

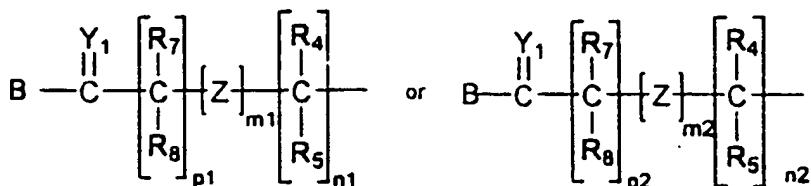
10



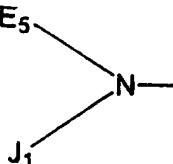
wherein:



$E_{1-4}$  are independently selected from the group consisting of hydrogen,  $C_{1-6}$  alkyls,  $C_{3-12}$  branched alkyls,  $C_{3-8}$  cycloalkyls,  $C_{1-6}$  substituted alkyls,  $C_{3-8}$  substituted cycloalkyls, aryls, substituted aryls, aralkyls,  $C_{1-6}$  heteroalkyls, substituted  $C_{1-6}$  heteroalkyls,  $C_{1-6}$  alkoxy, phenoxy,  $C_{1-6}$  heteroalkoxy,



and at least one of  $E_{1-4}$  includes a B moiety, wherein B is a leaving group, OH, a residue of a hydroxyl- or amine-containing moiety or



wherein  $J_1$  is the same as  $J$ , or another member of the group defining  $J$ ; optionally when  $J_1$  is the same as  $J$ ,  $m_1, n_1$  and  $p_1$ , etc. are different from those used in  $J$ ; and  $E_1$  is the same as  $E_{1-4}$ , or another member of the group defining  $E_{1-4}$ ;

$Y_{1-2}$  are independently O or S;

5  $M$  is a heteroatom selected from either X or Q; wherein X is an electron withdrawing group and Q is a moiety containing a free electron pair positioned three to six atoms from C(=Y<sub>2</sub>);

10  $R_{2-5}$  and  $R_{7-8}$  are independently selected from the group consisting of hydrogen, C<sub>1-6</sub> alkyls, C<sub>3-12</sub> branched alkyls, C<sub>3-8</sub> cycloalkyls, C<sub>1-6</sub> substituted alkyls, C<sub>3-8</sub> substituted cycloalkyls, aryls, substituted aryls, aralkyls, C<sub>1-6</sub> heteroalkyls, substituted C<sub>1-6</sub> heteroalkyls, C<sub>1-6</sub> alkoxy, phenoxy and C<sub>1-6</sub> heteroalkoxy;

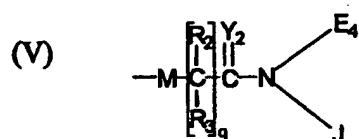
(m1) and (m2) are independently zero or one;

(n1), (n2), (p1), (p2) and (q) are independently zero or a positive integer;

Z is an electron withdrawing group; and

15  $R_1$  is a polymeric residue.

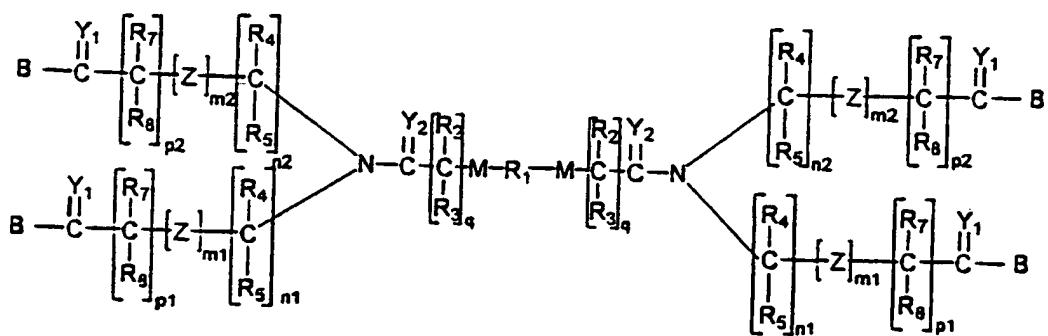
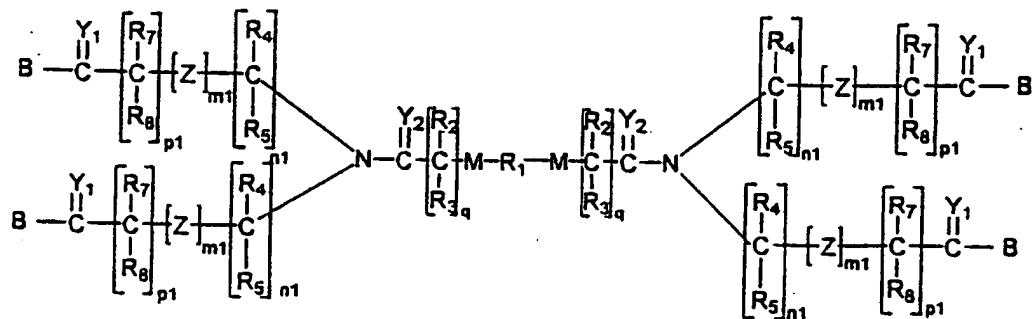
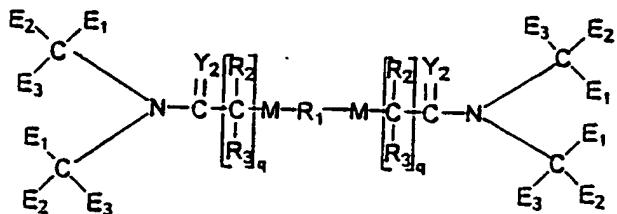
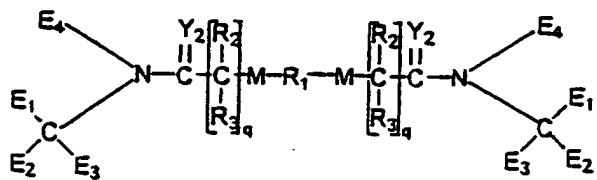
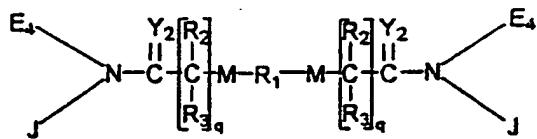
Preferably, the polymer residue portion, designated  $R_1$  herein, is further substituted with a terminal capping moiety (A<sub>1</sub>) which is distal to the linker portion containing the branched amine. A non-limiting list of suitable capping groups includes hydrogen, CO<sub>2</sub>H, C<sub>1-6</sub> alkyl moieties, biologically active and inactive moieties, dialkyl acyl urea alkyls, and moieties of Formula (V):

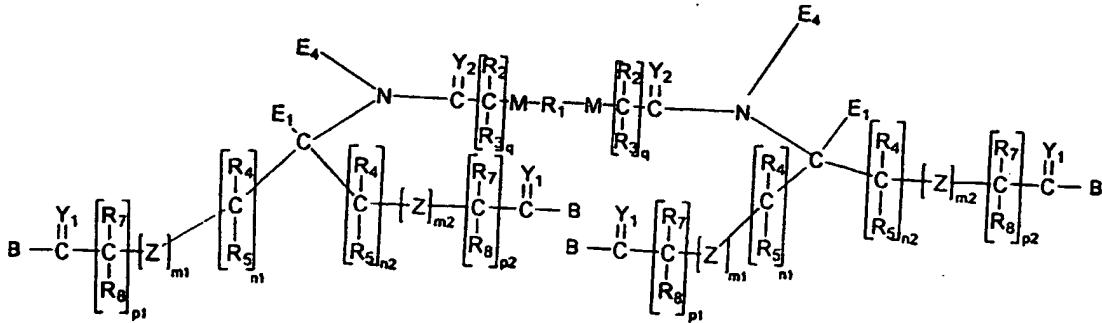


wherein all variables are as defined above.

Within Formula (I), Y<sub>1</sub> and Y<sub>2</sub> are preferably oxygen, R<sub>2-5</sub> are preferably H or methyl, (n) is 1 or 2, (p) is 1 and (q) is 1.

25 In those aspects of this embodiment where bis-substituted polymeric residues are desired, some preferred polymeric transport systems of the invention are shown below:





where all variables are as previously defined.

## B. THE PRODRUG LINKAGE

### 1. The Electron Withdrawing Groups X and Z

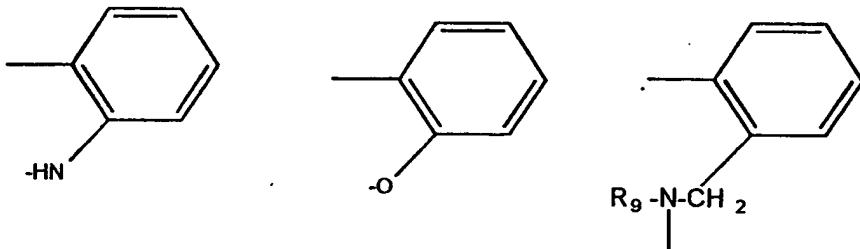
Within the Formula (I), X and Z are variables which represent electron withdrawing groups. In particular, X and Z can be independently selected from moieties such as O, S, SO, SO<sub>2</sub>, C(=Y<sub>3</sub>) wherein Y<sub>3</sub> is either O or S, and NR<sub>6</sub> wherein R<sub>6</sub> is one of hydrogen, C<sub>1-6</sub> alkyls, C<sub>3-12</sub> branched alkyls, C<sub>3-8</sub> cycloalkyls, C<sub>1-6</sub> substituted alkyls, C<sub>3-8</sub> substituted cycloalkyls, aryls, substituted aryls, aralkyls, C<sub>1-6</sub> heteroalkyls and substituted C<sub>1-6</sub> heteroalkyls, branched alkyls, aryls, substituted aryls, C<sub>1-6</sub> alkyl aralkyls, heteroalkyls, substituted heteroalkyls or substituted C<sub>1-6</sub> alkyls such as carboxyalkyls, aminoalkyls, dialkylaminos, hydroxyalkyls or mercaptoalkyls, to name but a few. Preferably, X is either O or NR<sub>6</sub> and R<sub>6</sub> is preferably H. In preferred embodiments, when X is oxygen, the oxygen is provided as the terminal portion of the PEG polymer. The terminal oxygen can be substituted to provide the other X moieties described herein using techniques apparent to those of ordinary skill without undue experimentation.

### 2. Q Portion of the Linker

When M is Q, the polymer, R<sub>1</sub>, is preferably attached to Q via a heteroatom such as oxygen. Q is a moiety containing a free electron pair positioned three to six atoms from the C(=Y<sub>2</sub>) moiety. In a preferred embodiment, the free electron pair is five atoms from the C(=Y<sub>2</sub>). Q can be selected from the non-limiting list of

cycloalkyls, aryls, aralkyl groups substituted with O, S or NR<sub>9</sub>, where R<sub>9</sub> is one of hydrogen, C<sub>1-6</sub> alkyls, C<sub>3-12</sub> branched alkyls, C<sub>3-8</sub> cycloalkyls, C<sub>1-6</sub> substituted alkyls, C<sub>3-8</sub> substituted cycloalkyls, aryls, substituted aryls, aralkyls, C<sub>1-6</sub> heteroalkyls and substituted C<sub>1-6</sub> heteroalkyls; -CH<sub>2</sub>-C(=O)-NH-, and ortho-substituted phenyls such as

5



Preferably, R<sub>9</sub> is H, a C<sub>1-6</sub> alkyl or substituted C<sub>1-6</sub> alkyl. The free electron pair can be anywhere along the Q moiety as long as the defined spacing between the free electron pair and the oxygen is maintained. In these embodiments, R<sub>1</sub> is attached to Q via NR<sub>9</sub>, O, or S. Thus, Q assists hydrolysis of the prodrug linkage by anchimeric assistance because the free electron pair moiety can generate a three- to six-membered, but preferably five-membered, ring by-product upon hydrolysis of the preferably ester linkage.

10

### 3. Drug Generation Via Hydrolysis of the Prodrug

The prodrug compounds of the present invention are designed so that the t<sub>1/2</sub> of hydrolysis is < t<sub>1/2</sub> elimination in plasma.

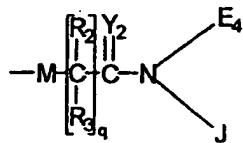
15

The linkages included in the compounds have hydrolysis rates in the plasma of the mammal being treated which is short enough to allow sufficient amounts of the parent compounds, i.e. the amino- or hydroxyl-containing bioactive compound, to be released prior to elimination. Some preferred compounds of the present invention, i.e. those in which (n) is 1, have a t<sub>1/2</sub> for hydrolysis in plasma ranging from about 5 minutes to about 12 hours. Preferably, the compositions have a plasma t<sub>1/2</sub> hydrolysis ranging from about 0.5 to about 8 hours and most preferably from about 1 to about 6 hours.

**C. SUBSTANTIALLY NON-ANTIGENIC POLYMERS**

As stated above, R<sub>1</sub> is a polymeric residue which is preferably substantially non-antigenic. In preferred aspects of the invention, R<sub>1</sub> further includes a capping group A which can be hydrogen, CO<sub>2</sub>H, C<sub>1-6</sub> alkyl moieties, carboxyalkyl, dialkyl acyl urea alkyls, or a compound of formula (V) shown below which forms a bis-system:

5 (V)



wherein all variables are the same as defined above.

Suitable examples of such polymers include polyalkylene oxides such as polyethylene glycols. The general formula for PEG and its derivatives, i.e.



where (x) represents the degree of polymerization (i.e. from about 10 to about 2,300) or number of repeating units in the polymer chain and is dependent on the molecular weight of the polymer, (n) is zero or a positive integer, (A<sub>2</sub>) is a capping group as defined herein, i.e. an, amino, carboxy, halo, C<sub>1-6</sub> alkyl or other activating group and (A'<sub>2</sub>) is the same as (A<sub>2</sub>) or another (A<sub>2</sub>) moiety. Also useful are polypropylene glycols, branched PEG derivatives such as those described in commonly-assigned U.S. Patent No. 5,643,575, "star-PEG's" and multi-armed PEG's such as those described in Shearwater Polymers, Inc. catalog "Polyethylene Glycol Derivatives 1997-1998". The disclosure of each of the foregoing is incorporated herein by reference. It will be understood that the water-soluble polymer can be functionalized for attachment to the linkage via M, X or Q herein. As an example, the PEG portion of the inventive compositions can be one of the following non-limiting compounds:

25 -C(=Y)-(CH<sub>2</sub>)<sub>n</sub>-O-(CH<sub>2</sub>CH<sub>2</sub>O)<sub>x</sub>-A<sub>2</sub>, -C(=Y)-Y-(CH<sub>2</sub>)<sub>n</sub>-O-(CH<sub>2</sub>CH<sub>2</sub>O)<sub>x</sub>-A<sub>2</sub>,  
and -C(=Y)-NR<sub>6</sub>-(CH<sub>2</sub>)<sub>n</sub>-O-(CH<sub>2</sub>CH<sub>2</sub>O)<sub>x</sub>-A<sub>2</sub>,

where Y is O or S and A<sub>2</sub>, R<sub>6</sub>, (n) and (x) are as defined above.

In many aspects of the present invention, bis-activated polyethylene glycols are preferred when di-substituted polymer conjugates are desired. Alternatively,

polyethylene glycols (PEG's), mono-activated, C<sub>1-4</sub> alkyl-terminated PAO's such as mono-methyl-terminated polyethylene glycols (mPEG's) are preferred when mono-substituted polymers are desired.

In order to provide the desired hydrolyzable linkage, mono- or di-acid activated polymers such as PEG acids or PEG diacids can be used as well as mono- or di-PEG amines and mono- or di-PEG diols. Suitable PAO acids can be synthesized by first converting mPEG-OH to an ethyl ester followed by saponification. See also Gehrhardt, H., et al. Polymer Bulletin 18: 487 (1987) and Veronese, F.M., et al., J. Controlled Release 10; 145 (1989). Alternatively, the PAO-acid can be synthesized by converting mPEG-OH into a *t*-butyl ester followed by acid cleavage. See, for example, commonly assigned U.S. Patent No. 5,605,976. The disclosures of each of the foregoing are incorporated by reference herein.

Although PAO's and PEG's can vary substantially in number average molecular weight, polymers ranging from about 2,000 to about 100,000 are usually selected for the purposes of the present invention. Molecular weights of from about 5,000 to about 50,000 are preferred and 20,000 to about 40,000 are particularly preferred. The number average molecular weight of the polymer selected for inclusion in the prodrug must be sufficient so as to provide sufficient circulation of the prodrug before hydrolysis of the linker. Within the ranges provided above, polymers having molecular weight ranges of at least 20,000 are preferred for chemotherapeutic and organic moieties. In the case of some nucleophiles such as certain proteins, enzymes and the like, the number average molecular weight of the polymeric residue can range from about 2,000 to about 20,000.

The polymeric substances included herein are preferably water-soluble at room temperature. A non-limiting list of such polymers include polyalkylene oxide homopolymers such as polyethylene glycol (PEG) or polypropylene glycols, polyoxyethylenated polyols, copolymers thereof and block copolymers thereof, provided that the water solubility of the block copolymers is maintained.

As an alternative to PAO-based polymers, effectively non-antigenic materials such as dextran, polyvinyl alcohols, carbohydrate-based polymers,

hydroxypropylmethacrylamide (HPMA), and copolymers thereof etc. and the like can be used if the same type of activation is employed as described herein for PAO's such as PEG. Those of ordinary skill in the art will realize that the foregoing list is merely illustrative and that all polymeric materials having the qualities described herein are contemplated. For purposes of the present invention, "effectively non-antigenic" and "substantially non-antigenic" shall be understood to include all polymeric materials understood in the art as being substantially non-toxic and not eliciting an appreciable immune response in mammals.

It will be clear from the foregoing that other polyalkylene oxide derivatives of the foregoing, such as the polypropylene glycol acids, etc., as well as other bi-functional linking groups are also contemplated.

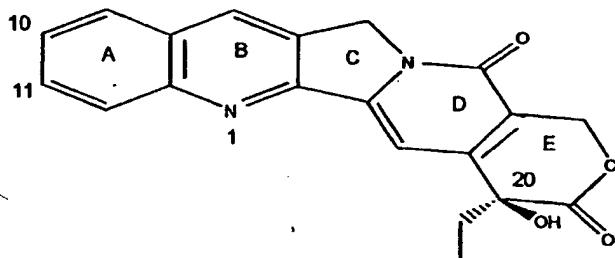
#### D. PRODRUG CANDIDATES

##### 1. Residues of Hydroxyl-containing Compounds

###### a. Camptothecin and Related Topoisomerase I Inhibitors

Camptothecin is a water-insoluble cytotoxic alkaloid produced by *Camptotheca acuminata* trees indigenous to China and *nothapodytes foetida* trees indigenous to India. Camptothecin and related compounds and analogs are also known to be potential anticancer or antitumor agents and have been shown to exhibit these activities *in vitro* and *in vivo*. Camptothecin and related compounds are also candidates for conversion to the prodrugs of the present invention.

Camptothecin and certain related analogues share the structure:



From this core structure, several known analogs have been prepared. For example, the A ring in either or both of the 10- and 11-positions can be substituted with an OH. The A ring can also be substituted in the 9-position with a straight or

branched C<sub>1-30</sub> alkyl or C<sub>1-17</sub> alkoxy, optionally linked to the ring by a heteroatom i.e.- O or S. The B ring can be substituted in the 7-position with a straight or branched C<sub>1-30</sub> alkyl or substituted alkyl-, C<sub>5-8</sub> cycloakyl, C<sub>1-30</sub> alkoxy, phenyl alkyl, etc., alkyl carbamate, alkyl carbazides, phenyl hydrazine derivatives, amino-, aminoalkyl-, aralkyl, etc. Other substitutions are possible in the C, D and E rings.

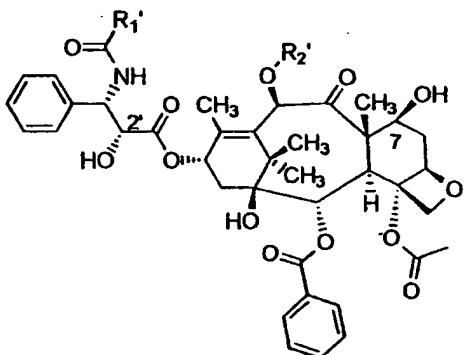
5 See, for example, U.S. Patent Nos. 5,004,758; 4,943,579; Re 32,518, the contents of which are incorporated herein by reference. Such derivatives can be made using known synthetic techniques without undue experimentation. Preferred camptothecin derivatives for use herein include those which include a 20-OH or another OH moiety which is capable of reacting directly with activated forms of the polymer transport systems described herein or to the linking moiety intermediates, e.g. iminodiacetic acid, etc., which are then attached to a polymer such as PEG.

10 Reference to camptothecin analogs herein has been made for purposes of illustration and not limitation.

15

b. Taxanes and Paclitaxel Derivatives

One class of compounds included in the prodrug compositions of the present invention is taxanes. For purposes of the present invention, the term "taxane" includes all compounds within the taxane family of terpenes. Thus, taxol (paclitaxel), 3'-substituted *tert*-butoxy-carbonyl-amine derivatives (taxoteres) and the like as well as other analogs which are readily synthesized using standard organic techniques or are available from commercial sources such as Sigma 20 Chemical of St. Louis, Missouri are within the scope of the present invention. Representative taxanes are shown below.



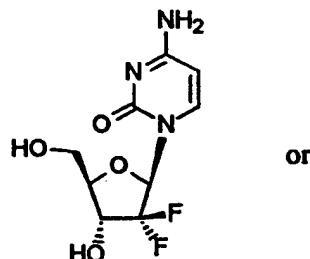
Paclitaxel: R<sub>1'</sub> = C<sub>6</sub>H<sub>5</sub>-, R<sub>2'</sub> = CH<sub>3</sub>C(=O)  
Taxotere: R<sub>1'</sub> = (CH<sub>3</sub>)<sub>3</sub>C(=O)-, R<sub>2'</sub> = H

These derivatives have been found to be effective anti-cancer agents.

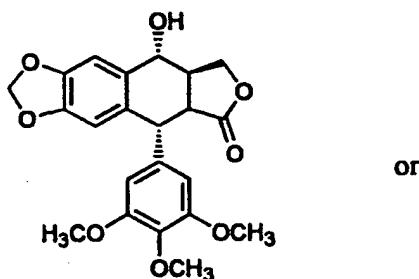
Numerous studies indicate that the agents have activity against several malignancies. To date, their use has been severely limited by, among other things, their short supply, poor water solubility and a tendency to cause hypersensitivity. It  
 5 is to be understood that other taxanes including the 7-aryl-carbamates and 7-carbazates disclosed in commonly assigned U.S. Patent Nos. 5,622,986 and 5,547,981 can also be included in the prodrugs of the present invention. The contents of the foregoing U.S. patents are incorporated herein by reference. The only limitation on the taxane is that it must be capable of undergoing a hydroxyl  
 10 based substitution reaction such as at the 2' position. Paclitaxel, however, is a preferred taxane.

c. Additional Biologically-Active Moieties

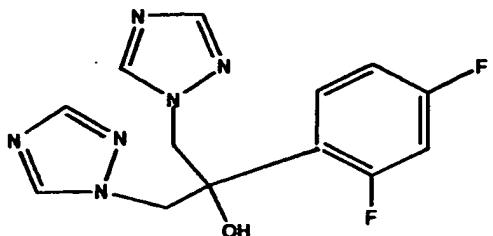
In addition to the foregoing molecules, the prodrug formulations of the present invention can be prepared using many other compounds. For example, biologically-active compounds such as bis-PEG conjugates derived from  
 15 compounds such as gemcitabine:



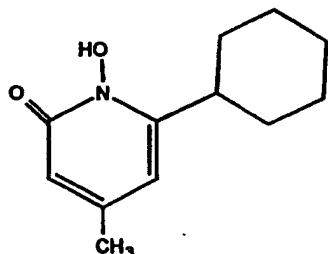
20 podophyllotoxin:



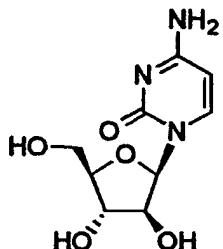
triazole-based antifungal agents such as fluconazole:



or ciclopirox:



or Ara-C:



5

The parent compounds selected for prodrug forms need not be substantially water-insoluble, although the polymer-based prodrugs of the present invention are especially well suited for delivering such water-insoluble compounds. Other useful parent compounds include, for example, certain low molecular weight biologically active proteins, enzymes and peptides, including peptido glycans, as well as other anti-tumor agents; cardiovascular agents such as forskolin; anti-neoplastics such as combretastatin, vinblastine, doxorubicin, maytansine, etc.; anti-infectives such as vancomycin, erythromycin, etc.; anti-fungals such as nystatin, amphotericin B, triazoles, papulocandins, pneumocandins, echinocandins, polyoxins, nikkomycins, pradimicins, benanomicins, etc. see, "Antibiotics That Inhibit Fungal Cell Wall Development" *Annu. Rev. Microbiol.* 1994, 48:471-97, the contents of which are

incorporated herein by reference; anti-anxiety agents; gastrointestinal agents, central nervous system-activating agents, analgesics, fertility or contraceptive agents, anti-inflammatory agents, steroidal agents, anti-urecemic agents, cardiovascular agents, vasodilating agents, vasoconstricting agents and the like.

The foregoing is illustrative of the biologically active moieties which are suitable for the prodrugs of the present invention. It is to be understood that those biologically active materials not specifically mentioned but having suitable ester-forming groups, i.e. hydroxyl moieties, are also intended and are within the scope of the present invention. It is also to be understood that the prodrug conjugates of the present invention may also include minor amounts of compounds containing not only one equivalent of drug and polymer but also a moiety which does not effect bioactivity *in vivo*. For example, it has been found that in some instances, in spite of reacting diacids with drug molecules having a single linkage point, the reaction conditions do not provide quantitative amounts of prodrugs with two equivalents of drug per polymer. By-products of the reactants can sometimes be formed such as acyl ureas if carbodiimides are used.

## 2. Residues of Amine-containing Compounds

In some aspects of the invention, B is a residue of an amine-containing compound, a non-limiting list of such suitable compounds include residues of organic compounds, enzymes, proteins, polypeptides, etc. Organic compounds include, without limitation, moieties such as anthracycline compounds including daunorubicin, doxorubicin; *p*-aminoaniline mustard, melphalan, Ara-C (cytosine arabinoside) and related anti-metabolite compounds, e.g., gemcitabine, etc.

Alternatively, B can be a residue of an amine-containing cardiovascular agent, anti-neoplastic, anti-infective, anti-fungal such as nystatin and amphotericin B, anti-anxiety agent, gastrointestinal agent, central nervous system-activating agent, analgesic, fertility agent, contraceptive agent, anti-inflammatory agent, steroidal agent, anti-urecemic agent, vasodilating agent, vasoconstricting agent, etc.

In a preferred aspect of the invention, the amino-containing compound is a biologically active compound that is suitable for medicinal or diagnostic use in the treatment of animals, e.g., mammals, including humans, for conditions for which

such treatment is desired. The foregoing list is meant to be illustrative and not limiting for the compounds which can be modified. Those of ordinary skill will realize that other such compounds can be similarly modified without undue experimentation. It is to be understood that those biologically active materials not specifically mentioned but having suitable amino-groups are also intended and are within the scope of the present invention.

The only limitations on the types of amino-containing molecules suitable for inclusion herein is that there is available at least one (primary or secondary) amine-containing position which can react and link with a carrier portion and that there is not substantial loss of bioactivity after the prodrug system releases and regenerates the parent compound.

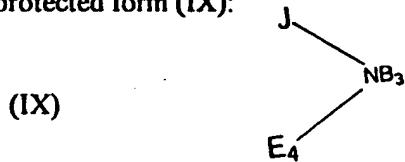
It is noted that parent compounds suitable for incorporation into the prodrug compositions of the invention, may themselves be substances/compounds which are not active after hydrolytic release from the linked composition, but which will become active after undergoing a further chemical process/reaction. For example, an anticancer drug that is delivered to the bloodstream by the double prodrug transport system, may remain inactive until entering a cancer or tumor cell, whereupon it is activated by the cancer or tumor cell chemistry, e.g., by an enzymatic reaction unique to that cell.

#### E. SYNTHESIS OF THE POLYMERIC PRODRUG TRANSPORT SYSTEM

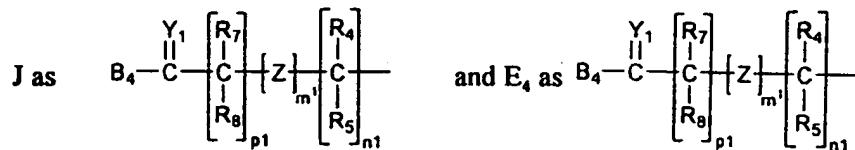
The prodrugs of the present invention can be prepared in at least two general fashions. In the first method, schematically illustrated in Figures 1a (symmetrical branches) and 1b (asymmetrical branches), the polymer residue is attached to the branching groups and thereafter the biologically active moiety or drug, e.g. Drug-OH or Drug-NH<sub>2</sub>, is attached to the polymeric terminal branches. In the second method, the biologically active moiety or drug, e.g. Drug-OH or Drug-NH<sub>2</sub>, is attached to the branching groups and thereafter, the resultant intermediate is attached to the polymeric residue. Figures 1a and 2a schematically illustrate methods in which symmetrical branches are used. Methods in which asymmetrical branches are used are illustrated in Figures 1b and 2b.

**1. First Method**

According to a first method, the branched amine-containing group is provided in a protected form (IX):



wherein  $E_4$  and  $J$  are as defined above and  $B_3$  is a cleavable or reversible protecting group. An example of a compound of formula (IX) is tBoc aspartic acid. Suitable protecting groups useful for this purpose may be any of a variety of organic moieties known to those of ordinary skill in the art and include, without limitation, t-Boc (*tert*-butyloxycarbonyl), Cbz (carbobenzyloxy) and TROC (trichloroethoxycarbonyl). In Figure 1a, compound (IXa) shows

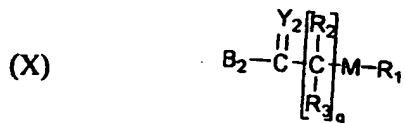


where  $B_4$  is OH and all other variables are as defined above.

The protecting group is removed by treatment of (IXa) with a strong acid such as trifluoroacetic acid (TFA) or other haloacetic acid, HCl, sulfuric acid, etc., or by using catalytic hydrogenation. The resulting unprotected amine terminal group (IXb1)



is then reacted with an activated polymer of Formula (X):

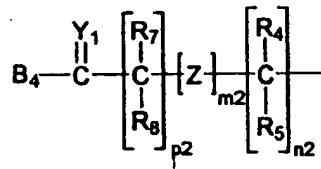


wherein  $M$ , (q) and  $R_{1,3}$  are as defined above,  $Y_2$  is O or S and  $B_2$  is a leaving group which is capable of reacting with an unprotected amine, such as an activated carbonate moiety like *p*-nitrophenyl or succinimidyl carbonate; a thiazolidine thione or other art recognized activating group to form (IXc). In the final synthesis step, a

biologically active moiety having an available OH or NH<sub>2</sub> group is reacted with (IXc) to form the polymeric transport form (IXd).

Attachment of the B moiety, e.g. Drug-OH or Drug-NH<sub>2</sub>, is preferably carried out in the presence of a coupling agent. A non-limiting list of suitable coupling agents include 1,3-diisopropylcarbodiimide (DIPC), any suitable dialkyl carbodiimides, 2-halo-1-alkyl-pyridinium halides, (Mukaiyama reagents), 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide (EDC), propane phosphonic acid cyclic anhydride (PPACA) and phenyl dichlorophosphates, etc. which are available, for example from commercial sources such as Sigma-Aldrich Chemical, or synthesized using known techniques.

Figure 1b shows a similar reaction scheme except that asymmetric branches are used, i.e. J is the same as in Figure 1a and E<sub>4</sub> is



where at least one of (m2), (n2) and (p2) is not the same as (m1), (n1) and (p1), respectively. While not illustrated in Figures 1a and 1b, the reaction scheme for attaching biologically active moieties having an available NH<sub>2</sub> group such as a protein or enzyme proceeds in a similar manner. All variables shown in the schematics of Figures 1a and 1b are the same as that previously defined herein.

Preferably the substituents are reacted in an inert solvent such as methylene chloride, chloroform, toluene, DMF or mixtures thereof. The reaction also preferably is conducted in the presence of a base, such as dimethylaminopyridine, diisopropylethylamine, pyridine, triethylamine, etc. to neutralize any acids generated and at a temperature from 0°C up to about 22°C (room temperature).

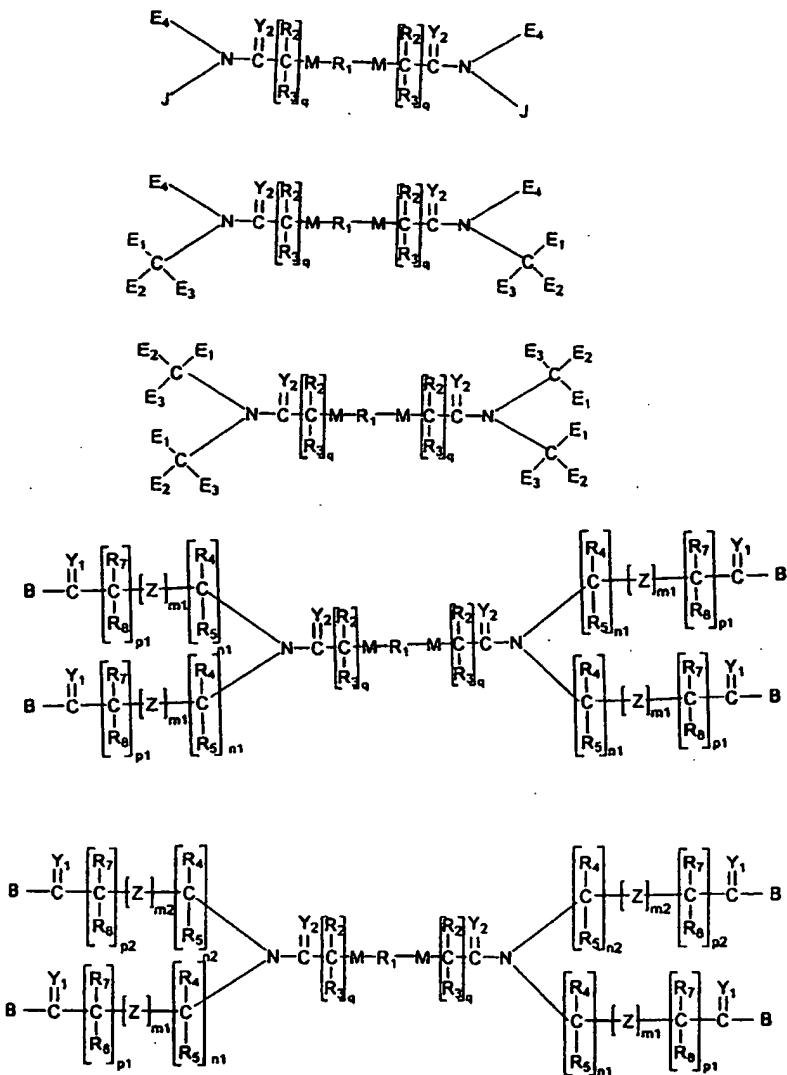
## 2. Second Method

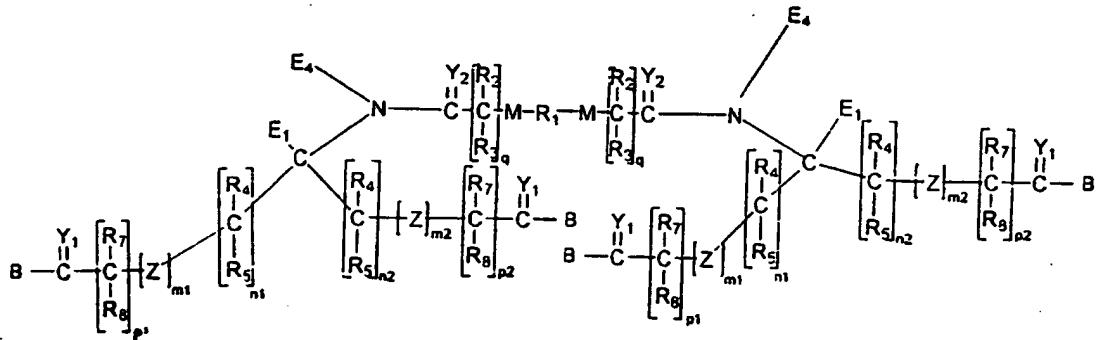
Turning now to the second method illustrated in Figures 2a and 2b, an alternative synthetic technique is shown using the same moieties for J and B<sub>4</sub> as that which was used in the first method. In this embodiment, the protected intermediate (IX) is reacted with a B moiety, e.g. Drug-OH as shown or Drug-NH<sub>2</sub>, prior to being deprotected. This results in the formation of a linkable ester-containing

5

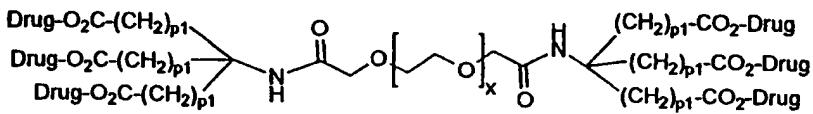
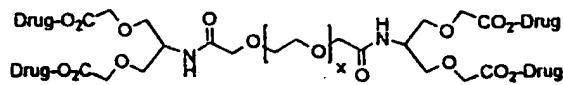
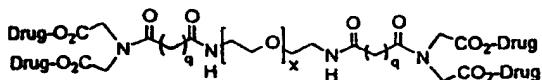
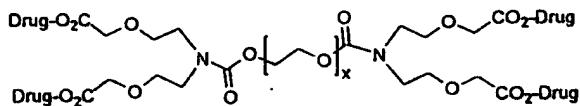
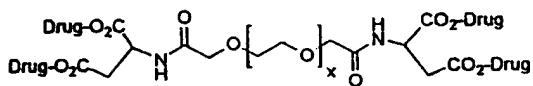
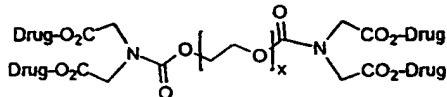
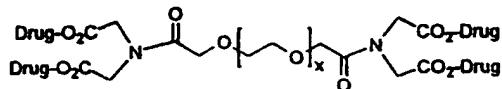
moiety (XI) which is then subjected to deprotecting to give (XIa) and polymer conjugating steps described above in the first method A to form the polymer transport form (IXd). Figure 2a shows the formation of symmetrical terminally branched conjugates while Figure 2b shows the formation of asymmetrical terminally branched conjugates. Amine-containing prodrugs can be made in the same way.

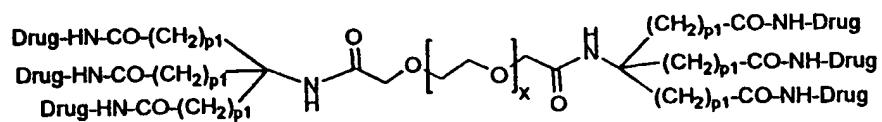
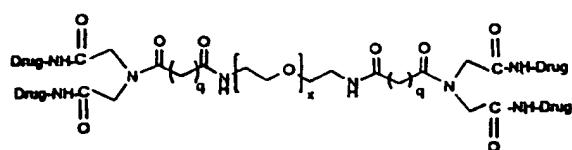
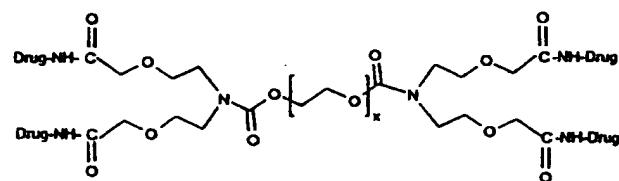
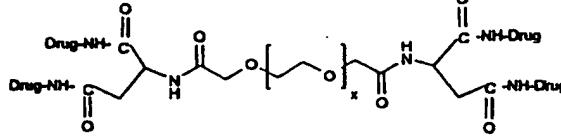
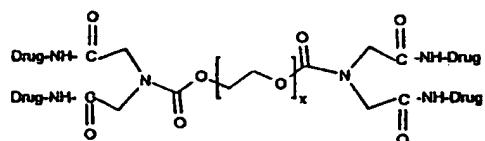
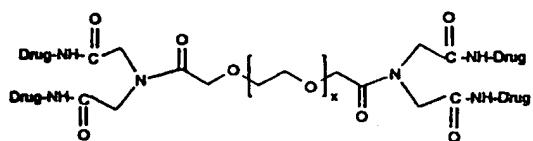
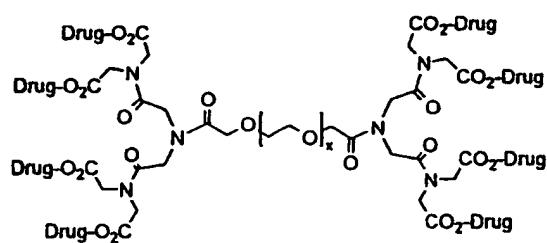
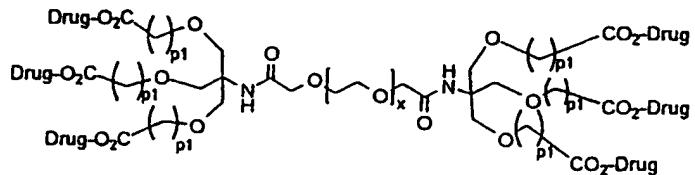
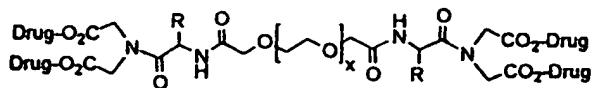
Regardless of the synthesis selected, some of the preferred compounds which result from the synthesis techniques described herein include:

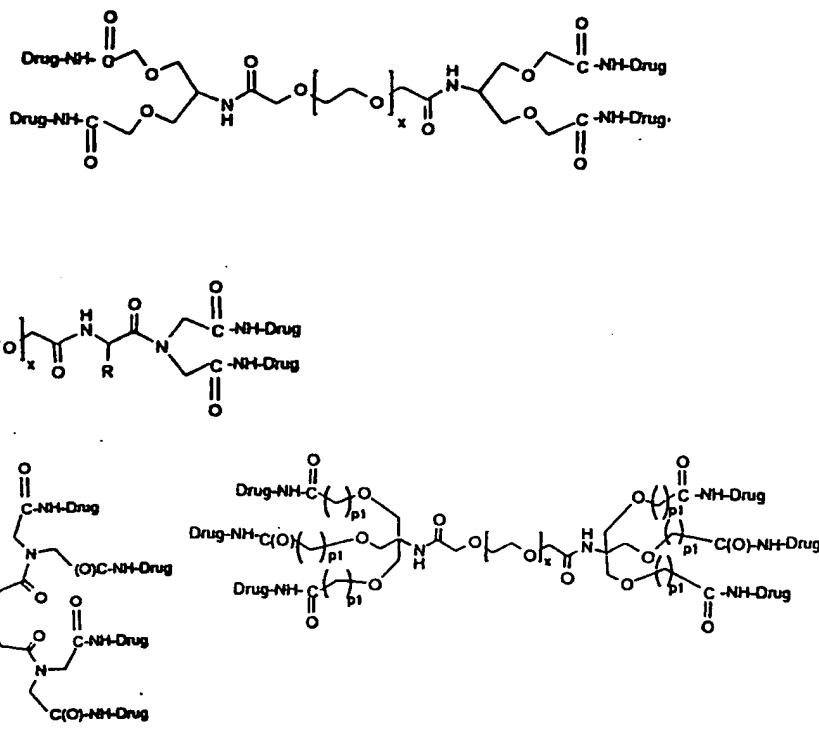




where all variables are as previously defined. In addition, some compounds prepared in accordance with preferred embodiments of the invention include:







wherein (x) represents the degree of polymerization and "Drug" represents a residue of a hydroxyl- or amine-containing biologically active compound which has undergone a substitution reaction which results in the attachment of the biologically active moiety to the branched polymer.

5

#### G. METHODS OF TREATMENT

Another aspect of the present invention provides methods of treatment for various medical conditions in mammals. The methods include administering to the mammal in need of such treatment, an effective amount of a prodrug, such as a camptothecin-20-PEG ester, which has been prepared as described herein. The compositions are useful for, among other things, treating neoplastic disease, reducing tumor burden, preventing metastasis of neoplasms and preventing recurrences of tumor/neoplastic growths in mammals.

10

The amount of the prodrug administered will depend upon the parent molecule included therein. Generally, the amount of prodrug used in the treatment methods is that amount which effectively achieves the desired therapeutic result in mammals. Naturally, the dosages of the various prodrug compounds will vary somewhat depending upon the parent compound, rate of in vivo hydrolysis,

molecular weight of the polymer, etc. In general, however, prodrug taxanes are administered in amounts ranging from about 5 to about 500 mg/m<sup>2</sup> per day, based on the amount of the taxane moiety. Camptothecin prodrugs are also administered in amounts ranging from about 5 to about 500 mg/m<sup>2</sup> per day. The range set forth above is illustrative and those skilled in the art will determine the optimal dosing of the prodrug selected based on clinical experience and the treatment indication.

5 Actual dosages will be apparent to the artisan without undue experimentation.

The prodrugs of the present invention can be included in one or more suitable pharmaceutical compositions for administration to mammals. The pharmaceutical compositions may be in the form of a solution, suspension, tablet, capsule or the like, prepared according to methods well known in the art. It is also contemplated that administration of such compositions may be by the oral and/or parenteral routes depending upon the needs of the artisan. A solution and/or suspension of the composition may be utilized, for example, as a carrier vehicle for injection or infiltration of the composition by any art known methods, e.g., by intravenous, intramuscular, subdermal injection and the like.

10

15

Such administration may also be by infusion into a body space or cavity, as well as by inhalation and/or intranasal routes. In preferred aspects of the invention, however, the prodrugs are parenterally administered to mammals in need thereof.

## 20 H. EXAMPLES

The following examples serve to provide further appreciation of the invention but are not meant in any way to restrict the effective scope of the invention. The underlined and bold-faced numbers recited in the Examples correspond to those shown in the Figures.

### 25 Example 1

#### Compound 2: N-t-Boc-iminodiacetic Acid

A mixture of iminodiacetic acid (**1**, 2 g, 15.03 mmol), di-t-butyl dicarbonate (3.9 g, 18.0 mmol), and sodium hydroxide (0.721 g, 18.0 mmol) in water (50 mL) was stirred at room temperature for 18 hours. The reaction solution was washed with 20 mL of methylene chloride (CH<sub>2</sub>Cl<sub>2</sub>) followed by adjusting pH to 2.5 with

30

6 N HCl. The resulting mixture was extracted with ethyl acetate (2 x 300 mL) and the combined organic layer was dried over anhydrous magnesium sulfate ( $MgSO_4$ ). The solvent was removed in vacuo to give 1.0 g (29%) of 2.

5        $^1H$  NMR (270 MHz, DMSO- $d_6$ )  $\delta$  1.36 (s, 9H), 3.88 (s, 2H), 3.92 (s, 2H), 13.69 (bs, 2H).  $^{13}C$  NMR (67.80 MHz, DMSO- $d_6$ )  $\delta$  27.84, 49.12, 49.64, 79.59, 154.79, 171.20.

**Example 2**

**Compound 4: Coupling of 2 with Camptothecin(3)**

10      A mixture of 2 (200 mg, 0.86 mmol) and camptothecin (3, 777 mg, 2.2 mmol) in anhydrous  $CH_2Cl_2$  (50 mL) was cooled in an ice bath for 30 minutes before adding 1,3-diisopropylcarbodiimide (DIPC, 324 mg, 2.4 mmol) and 4-dimethylaminopyridine (DMAP, 272 mg, 2.2 mmol). The reaction mixture was left in the ice bath overnight and was allowed to warm to room temperature slowly. The solution was filtered and washed with water (20 mL) and 1N HCl (20 mL).

15      The organic layer was dried over anhydrous  $MgSO_4$  and concentrated. The residue was purified by silica gel column chromatography (2.5% methanol in  $CH_2Cl_2$ ) to give 432 mg (56%) of 4.

20       $^1H$  NMR (270 MHz,  $CDCl_3$ )  $\delta$  1.00 (t, 6H,  $J$  = 8.1 Hz), 1.20 (s, 3H), 1.22 (s, 3H), 1.38 (s, 3H), 1.44 (s, 3H), 2.17 (m, 4H), 4.01-4.36 (m, 4H), 5.26 (d, 2H,  $J$  = 13.5 Hz), 5.38 (d, 2H,  $J$  = 10.1 Hz), 5.41 (d, 2H,  $J$  = 5.4 Hz), 5.24 (d, 2H,  $J$  = 8.1 Hz), 7.25 (d, 2H,  $J$  = 13.5 Hz), 7.37 (s, 2H), 7.62 (t, 2H,  $J$  = 8.1 Hz), 7.79 (q, 2H,  $J$  = 8.1 Hz), 7.90 (m, 2H), 8.19 (m, 2H), 8.35 (d, 2H,  $J$  = 10.8 Hz).

25       $^{13}C$  NMR (67.80 MHz,  $CDCl_3$ )  $\delta$  7.59, 22.20, 23.37, 25.35, 28.07, 31.57, 31.75, 49.37, 49.56, 49.97, 64.38, 66.94, 74.94, 76.76, 76.79, 78.82, 81.74, 95.83, 96.69, 119.75, 120.12, 127.86, 128.04, 128.15, 128.36, 129.59, 130.40, 130.60, 130.87, 131.10, 145.65, 145.84, 146.31, 146.40, 148.86, 152.14, 152.30, 154.81, 157.34, 166.83, 167.25, 168.78, 169.07.

**Example 3**

**Compound 5: Deprotection of 4**

30      A solution of 4 (300 mg, 0.34 mmol) in anhydrous  $CH_2Cl_2$  (5 mL) and trifluoroacetic acid (TFA, 2.5 mL) was stirred at room temperature for 3 hours.

The reaction mixture was concentrated and the solid was recrystallized from ethyl ether to give 258 mg (78%) of 5 as a TFA salt.

<sup>1</sup>H NMR (270 MHz, DMSO-*d*<sub>6</sub>) δ 0.92 (t, 6H, *J* = 8.1 Hz), 2.16 (q, 4H, *J* = 8.1 Hz), 4.19 (d, 2H, *J* = 16.2 Hz), 4.36 (d, 2H, *J* = 16.2 Hz), 5.26 (s, 4H), 5.54 (s, 4H), 7.22 (s, 2H), 7.71 (t, 2H, *J* = 8.1 Hz), 7.84 (t, 2H, *J* = 8.1 Hz), 8.10 (s, 2H), 8.13 (s, 2H), 8.67 (s, 2H). <sup>13</sup>C NMR (67.80 MHz, DMSO-*d*<sub>6</sub>) δ 7.47, 30.08, 38.58, 38.88, 39.19, 39.50, 39.81, 40.12, 40.42, 46.81, 50.19, 66.32, 77.37, 95.13, 118.79, 127.71, 127.92, 128.54, 128.68, 129.69, 130.45, 131.62, 144.65, 146.03, 147.80, 152.21, 156.41, 166.76.

10 **Example 4**

**Compound 7: PEGylation of 5**

PEG (40 kDa) dicarboxylic acid (6, 2.0 g, 0.05 mmol) was azeotroped for 2 hours in toluene, followed by removal of the solvent *in vacuo*. Anhydrous CH<sub>2</sub>Cl<sub>2</sub> (20 mL) was added to the residue followed by the addition of 5 (0.16 g, 0.20 mmol), DIPC (25 mg, 0.20 mmol), and DMAP (25 mg, 0.20 mmol). The reaction mixture was stirred at room temperature overnight followed by removal of the solvent *in vacuo*. The residue was recrystallized from 2-propanol to yield 0.8 g (69%) of 7 as a white solid.

<sup>13</sup>C NMR (67.80 MHz, CDCl<sub>3</sub>) δ 7.25, 31.43, 49.35, 49.64, 66.80, 68.66-71.16 (PEG), 76.06, 95.57, 119.96, 127.71, 127.89, 128.13, 129.38, 130.34, 130.89, 145.11, 146.09, 148.54, 151.93, 156.94, 166.89, 170.58.

15 **Example 5**

**Compound 9: Coupling of 1 with PEG (40 kDa) Dithiazolidine Thione (8)**

PEG (40 kDa) dithiazolidine thione (8, 1 g, 0.025 mmol) is added to the mixture of 1 (14 mg, 0.11 mmol) and *N,N*-diisopropylethylamine (DIPEA, 37 μL, 0.20 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (15 mL). The mixture is stirred at room temperature overnight. The solvent is removed under reduced pressure and the residue is recrystallized from 2-propanol to give 9.

20 **Example 6**

**Compound 7 from Compound 9**

DIPC (13 mg, 0.10 mmol) is added to the mixture of 9 (1.0 g, 0.025 mmol),

5 DMAP (13 mg, 0.10 mmol), and 3 (35 mg, 0.1 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (20 mL). The solution is stirred at room temperature overnight followed by removal of the solvent *in vacuo*. The residue is recrystallized from 2-propanol (80 mL) to give 7.

10 **Example 7**

**Compound 11: *N*-t-Boc-diethanolamine**

15 A solution of di-*t*-butyl dicarbonate (26.46 g, 0.12 mol) in chloroform (50 mL) was added to the solution of diethanolamine (10, 12.63 g, 0.12 mol) in chloroform (50 mL) slowly at room temperature. The reaction solution was stirred at room temperature for 1 hour, followed by washing with water (30 mL) and the organic layer was dried over anhydrous MgSO<sub>4</sub>. The solvent was removed under reduced pressure to give 11 (20 g, 83%).

<sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) δ 1.46 (s, 9H), 3.41 (bs, 4H), 3.76 (bs, 4H), 4.69 (bs, 2H). <sup>13</sup>C NMR (67.80 MHz, CDCl<sub>3</sub>) δ 28.30, 52.22, 61.63, 80.13, 156.22.

20 **Example 8**

**Compound 12**

25 Compound 11 (9.5 g, 46.34 mmol) was dissolved in anhydrous toluene (200 mL) by warming and the solution cooled to -20 °C followed by the addition of potassium *t*-butoxide (1M solution in *t*-butanol, 70 mL, 70 mmol). The mixture was stirred at -20 °C for 5 hours and then cooled to -30 °C. Ethyl bromoacetate (30.96 g, 185.35 mmol) was added to the solution and the reaction mixture was stirred at -15 °C for 3 hours. The solution was washed with water (50 mL) and the organic layer was dried over anhydrous MgSO<sub>4</sub>. The solvent was removed *in vacuo* to give a crude product which was purified by silica gel column chromatography (ethyl acetate/hexane = 1:1, v/v) to give 8.2 g (48%) of 12.

30 <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) δ 1.28 (t, 6H, *J* = 5.4 Hz), 1.45 (s, 9H), 3.51 (bs, 4H), 3.67 (bs, 4H), 4.08 (s, 4H), 4.21 (q, 4H, *J* = 5.4 Hz). <sup>13</sup>C NMR (67.80 MHz, CDCl<sub>3</sub>) δ 13.95, 28.15, 47.61, 60.49, 68.16, 69.96, 79.42, 155.14, 170.02.

**Example 9****Compound 13**

A solution of NaOH (10 g, 250 mmol) in water (10 mL) and ethanol (100 mL) was added to a solution of 12 (8.0 g, 21.22 mmol) in ethanol (80 mL). The reaction solution was stirred at room temperature for 1.5 hours and cooled to 0 °C. The pH was adjusted to 2.5 with 6N HCl. The mixture was filtered and the filtrate was concentrated *in vacuo*. Chloroform (300 mL) was added to the residue and washed with water (3 x 50 mL). The organic layer was dried over anhydrous MgSO<sub>4</sub> and concentrated under reduced pressure to give 5.0 g (73%) of 13.

<sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) δ 1.45 (s, 9H), 3.51 (bs, 4H), 3.71 (bs, 4H), 4.13 (s, 4H), 9.35 (bs, 2H). <sup>13</sup>C NMR (67.80 MHz, CDCl<sub>3</sub>) δ 28.35, 48.13, 67.97, 70.24, 80.54, 155.93, 173.95.

**Example 10****Compound 14: Coupling of 13 with 3**

A mixture of 13 (2 g, 6.23 mmol), 3 (5.643 g, 16.20 mmol), DMAP (1.979 g, 16.20 mmol), and DIPC (2.041 g, 16.20 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (50 mL) was stirred at room temperature for 20 hours. The reaction mixture was filtered and the filtrate washed with water (30 mL) and dried over anhydrous MgSO<sub>4</sub>. The solution was concentrated to give crude product which was purified by silica gel column chromatography (2.5 % methanol in CH<sub>2</sub>Cl<sub>2</sub>) to give 14 as a light yellow solid (2.45 g, 40%).

<sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) δ 0.96 (t, 6H, *J* = 8.1 Hz), 1.38 (s, 9H), 2.20 (qd, 4H, *J* = 13.5, 8.1 Hz), 3.47 (bs, 4H), 3.63 (bs, 4H), 4.25 (s, 4H), 5.24 (s, 4H), 5.39 (d, 2H, *J* = 13.5 Hz), 5.66 (d, 2H, *J* = 13.5 Hz), 7.19 (s, 2H), 7.65 (t, 2H, *J* = 6.8 Hz), 7.80 (t, 2H, *J* = 6.8 Hz), 7.93 (d, 2H, *J* = 8.1 Hz), 8.2 (d, 2H, *J* = 8.1 Hz), 8.36 (s, 2H). <sup>13</sup>C NMR (67.80 MHz, CDCl<sub>3</sub>) δ 7.46, 28.27, 31.70, 47.53, 47.74, 49.83, 67.06, 67.84, 70.37, 76.22, 79.68, 95.76, 120.19, 127.92, 128.07, 128.36, 129.53, 130.55, 131.07, 145.29, 146.30, 148.71, 152.15, 155.25, 157.18, 167.09, 169.36.

**Example 11****Compound 15: Deprotection of 14**

Compound **14** (0.74 g, 0.75 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) and TFA (5 mL). The reaction solution was stirred at room temperature for 2 hours and concentrated under reduced pressure. The residue was recrystallized from CH<sub>2</sub>Cl<sub>2</sub>-ethyl ether to give 0.6 g (100%) of **15** as a TFA salt.

<sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) δ 0.9, 2.1, 3.3, 3.9, 4.4, 5.2, 5.4, 5.6, 7.2, 7.6, 7.8, 7.9, 8.1, 8.4. <sup>13</sup>C NMR (67.80 MHz, CDCl<sub>3</sub>) δ 7.17, 31.20, 46.85, 49.77, 53.40, 66.43, 67.61, 76.79, 95.46, 119.51, 127.70, 127.94, 128.43, 129.04, 130.36, 131.05, 145.00, 146.23, 148.29, 151.86, 156.87, 166.85, 169.49.

10

**Example 12****Compound 16: Coupling of 15 with Di-SC-PEG (40 kDa)**

A mixture of **15** (79.8 mg, 0.09 mmol), di-SC-PEG (40 kDa, 1.0 g, 0.025 mmol), and DMAP (11.1 mg, 0.09 mmol) in anhydrous chloroform (20 mL) was stirred at room temperature overnight. The solvent was removed *in vacuo* and the residue was recrystallized from 2-propanol (80 mL) to give 0.92 g (92%) of **16**.

<sup>13</sup>C NMR (67.80 MHz, CDCl<sub>3</sub>) δ 7.15, 31.54, 47.68, 49.63, 64.17, 66.73, 67.92, 69.13-71.28 (PEG), 76.16, 95.33, 120.01, 127.57, 127.83, 127.97, 128.39, 129.45, 130.15, 130.71, 145.28, 146.22, 148.70, 152.12, 155.74, 156.94, 166.52, 168.90.

20

**Example 13****Compound 17: Coupling of 15 with PEG (40 kDa) Dicarboxylic Acid (6)**

Compound **6** (3 g, 0.075 mmol) was azeotroped for 2 hours in 90 mL of toluene. The solvent was removed *in vacuo* and the residue was dissolved in 50 mL of anhydrous CH<sub>2</sub>Cl<sub>2</sub>. Compound **15** (263.5 mg, 0.3 mmol), DMAP (45.7 mg, 0.38 mmol), and DIPC (37.7 mg, 0.30 mmol) were added to the solution and the reaction mixture was stirred at room temperature overnight. The reaction solution was diluted with 100 mL of CH<sub>2</sub>Cl<sub>2</sub> and washed with 1N HCl (2 x 20 mL) and water (20 mL). The organic layer was dried over anhydrous MgSO<sub>4</sub>, and the solvent was removed under reduced pressure. The residue was recrystallized from 2-propanol (100 mL) to give 2.44 g (80%) of **17**.

30

<sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>) δ 1.01, 2.2, 2.9, 3.2-3.9 (PEG), 4.2, 5.2, 5.3, 5.7, 7.15, 7.65, 7.8, 7.95, 8.2, 8.5. <sup>13</sup>C NMR (67.80 MHz, CDCl<sub>3</sub>) δ 6.91, 31.01, 45.05, 47.50, 49.33, 66.36, 67.21, 67.30-71.16 (PEG), 75.62, 75.72, 77.92, 94.86, 119.31, 119.38, 127.31, 127.53, 127.63, 127.99, 128.85, 129.93, 130.74, 144.61, 145.83, 148.05, 151.54, 156.45, 166.34, 168.47, 168.55, 169.23.

5

**Example 14**

**Compound 20:** Coupling of TRIS (18) with mPEG (20 kDa) Thiazolidine Thione (19).

10

mPEG (20 kDa) thiazolidine thione (19, 4 g, 0.2 mmol) was added to a solution of tris(hydroxymethyl)aminomethane (TRIS, 18, 2.4 g, 20 mmol) in water (60 mL). The mixture was stirred at room temperature overnight, followed by extraction with CH<sub>2</sub>Cl<sub>2</sub> (2 x 50 mL). The combined organic layer was washed with brine (60 mL) and dried over anhydrous MgSO<sub>4</sub>. The solvent was removed under reduced pressure and the residue was recrystallized from 2-propanol to give 2.0 g (50%) of 20.

15

<sup>13</sup>C NMR (67.80 MHz, CDCl<sub>3</sub>) δ 58.33, 60.99, 62.31, 69.91-71.28 (PEG), 170.51.

**Example 15**

**Compound 21**

20

A solution of 20 (10 g, 2 mmol) in 100 mL of toluene is azeotroped for 2 hours and is cooled to 35 °C followed by the addition of 10.5 mL (10.5 mmol) of 1.0 M potassium *t*-butoxide in *t*-butanol. The mixture is stirred for 1 hour at 35 °C followed by the addition of 3.9 g (20 mmol) of *t*-butyl bromoacetate. The reaction mixture is stirred at 40 °C overnight, filtered through celite and solvent removed *in vacuo*. The residue is recrystallized from chilled CH<sub>2</sub>Cl<sub>2</sub>-ethyl ether to yield ester of 20. The ester is dissolved in CH<sub>2</sub>Cl<sub>2</sub> (100 mL) and TFA (50 mL). The reaction solution is stirred at room temperature for 2 hours and concentrated under reduced pressure. The residue is recrystallized from CH<sub>2</sub>Cl<sub>2</sub>-ethyl ether to give 21.

25

**Example 16****Compound 22: Coupling of 3 with 21**

Compound 21 (3 g, 0.56 mmol) is azeotroped for 2 hours in 90 mL of toluene. The solvent is removed *in vacuo* and the residue is dissolved in 60 mL of anhydrous CH<sub>2</sub>Cl<sub>2</sub>. Compound 3 (1.17 g, 3.4 mmol), DMAP (829.6 mg, 6.8 mmol), and DIPC (1.37 g, 13.6 mmol) are added to the solution and the reaction mixture is stirred at room temperature overnight. The reaction solution is diluted with 100 mL of CH<sub>2</sub>Cl<sub>2</sub> and washed with 1N HCl (2 x 20 mL) and water (20 mL). The organic layer is dried over anhydrous MgSO<sub>4</sub> and the solvent is removed under reduced pressure. The residue is recrystallized from 2-propanol (300 mL) to give 22.

**Example 17****Compound 24: Coupling of 3 with N-t-Boc-L-Aspartic Acid (23)**

DIPC (0.72 g, 5.8 mmol) was added to a solution of *N*-t-Boc-L-aspartic acid (23, 1.34 g, 5.8 mmol), 3 (2.0 g, 5.8 mmol), DMAP (0.7 g, 5.8 mmol), and in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (25 mL) at 0 °C. The mixture was allowed to warm to room temperature overnight, followed by washing with 1% aqueous sodium bicarbonate (4 x 15 mL) and 0.1 N HCl (2 x 15 mL). The organic layer was dried over anhydrous MgSO<sub>4</sub>. The solution was concentrated to give a crude product as a solid which was recrystallized in methanol to give 24 (2.1 g, 40%).

<sup>13</sup>C NMR (67.80 MHz, CDCl<sub>3</sub>) δ 7.25, 7.47, 27.20, 27.83, 28.12, 31.30, 31.48, 35.66, 49.74, 66.46, 66.83, 80.11, 96.25, 96.57, 119.64, 119.86, 127.79, 127.91, 128.17, 128.36, 129.48, 129.59, 130.40, 130.92, 145.20, 145.84, 146.05, 148.59, 151.89, 152.07, 155.18, 156.84, 156.92, 166.51, 167.22, 169.68, 169.90.

**Example 18****Compound 25: Deprotection of 24**

A solution of 24 (500 mg, 0.56 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (5 mL) and trifluoroacetic acid (TFA, 2.5 mL) was stirred at room temperature for 1 hour, followed by addition of ethyl ether (40 mL). The solid was filtered and washed with ethyl ether to give 25 (0.4 g, 75%).

**Example 19****Compound 26: Coupling of 25 with 6**

PEG (40 kDa) dicarboxylic acid (**6**, 1.0 g, 0.025 mmol) was azeotroped for 2 h in toluene, followed by removal of the solvent *in vacuo*. Anhydrous CH<sub>2</sub>Cl<sub>2</sub> (20 mL) was added to the residue followed by the addition of **25** (94 mg, 0.10 mmol), DIPC (13 mg, 0.10 mmol), and DMAP (25 mg, 0.20 mmol). The reaction mixture was stirred at room temperature overnight followed by removal of the solvent *in vacuo*. The residue was recrystallized from 2-propanol to yield 0.81 g (81%) of **7** as a white solid.

5

10

**Example 20****Compound 28**

A mixture of **6** (5 g, 0.125 mmol), L-aspartic acid dimethylester HCl (**27**, 98.5 mg, 0.50 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, 191 mg, 1.0 mmol), and DMAP (122 mg, 1.0 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (80 mL) was stirred at room temperature overnight. The solvent was removed and the residue was recrystallized from 2-propanol to give 4.2 g (84%) of **28**.

15

<sup>13</sup>C NMR (67.80 MHz, CDCl<sub>3</sub>) δ 35.08, 47.08, 51.00, 51.72, 62.00-71.25 (PEG), 168.87, 169.85, 170.01.

20

**Example 21****Compound 29**

Compound **28** (3.8 g, 0.094 mmol) and lithium hydroxide (24 mg, 0.57 mmol) were stirred in water (20 mL) for 6 hours, followed by acidification with 1N HCl to adjust the pH to 3. The product was extracted to CH<sub>2</sub>Cl<sub>2</sub> and recrystallized with chilled CH<sub>2</sub>Cl<sub>2</sub>-ether to give 3.5 g (92%) of **29**.

25

<sup>13</sup>C NMR (67.80 MHz, CDCl<sub>3</sub>) δ 34.96, 46.80, 67.64-70.72 (PEG), 168.74, 170.35, 170.61.

**Example 22****Compound 31**

A mixture of 29 (1.0 g, 0.025 mmol), paclitaxel (30, 170 mg, 0.20 mmol), EDC (76 mg, 0.4 mmol), and DMAP (76 mg, 0.6 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (20 mL) was stirred at room temperature for 16 hours. The mixture was concentrated *in vacuo* and the residue was recrystallized from 2-propanol to give 0.95 g (95%) of 31.

<sup>13</sup>C NMR (67.80 MHz, CDCl<sub>3</sub>) δ 8.92, 13.75, 13.95, 20.08, 21.34, 21.84, 22.12, 34.75, 35.43, 42.47, 45.33, 47.09, 51.49, 51.64, 57.59, 69.80-71.10 (PEG), 74.26, 74.70, 75.56, 75.80, 77.84, 77.97, 80.31, 83.58, 125.65, 125.82, 126.25, 126.74, 126.84, 127.78, 128.00, 128.18, 128.54, 128.75, 129.45, 131.15, 132.38, 132.54, 132.72, 132.84, 135.59, 136.27, 141.11, 165.79, 165.86, 166.49, 167.59, 167.69, 169.16, 169.26, 169.41, 169.93, 202.70.

**Example 23****Compound 34**

A mixture of *t*-Boc-β-Alanine (32, 200 mg, 1.06 mmol), 2-mercaptop-thiazolidine (33, 252 mg, 2.12 mmol), EDC (407 mg, 2.12 mmol), and DMAP (516 mg, 4.23 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (10 mL) is stirred at room temperature overnight. The reaction mixture is diluted with 40 mL of CH<sub>2</sub>Cl<sub>2</sub> and washed twice with 1% NaHCO<sub>3</sub> (25 mL) and twice with 1N HCl. The organic layer is dried over anhydrous sodium sulfate and the solvent is removed *in vacuo* to give 34.

**Example 24****Compound 36**

A mixture of 34 (1.79 g, 6.17 mmol) and 35 (4.5 g, 18.5 mmol) in anhydrous pyridine (125 mL) is stirred at 40 °C overnight. The reaction mixture is concentrated *in vacuo* and the residue is purified by column chromatography to give 36.

**Example 25****Compound 37**

Compound 36 (1 g) is dissolved in TFA (10 mL) and CH<sub>2</sub>Cl<sub>2</sub> (10 mL) and the solution stirred at room temperature for 2 hours. The solvent is removed *in vacuo* and the product precipitated by adding anhydrous ethyl ether to give 37 as the TFA salt.

5

**Example 26****Compound 38**

A mixture of 29 (1.0 g, 0.025 mmol), 37 (106 mg, 0.20 mmol), EDC (76 mg, 0.4 mmol), and DMAP (76 mg, 0.6 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (20 mL) is stirred at room temperature for 16 hours. The mixture is concentrated *in vacuo* and the residue recrystallized from 2-propanol to give 38.

10

**Example 27****Compound 39**

15

In this example, a polymeric conjugate of gemcitabine is prepared by repeating the procedures of Examples 24-26 using gemcitabine in place of Ara-C (35).

20

**Example 28****Compound 41**

PEG 40 kDa diamine hydrochloride (5 g, 0.125 mmol) is dissolved in anhydrous dichloromethane (50 mL) and EDC (95 mg, 0.49 mmol) and DMAP (95 mg, 0.78 mmol) are added. The solution is stirred for 3 hours at room temperature. The dianhydride of diethylenetriaminepentaacetic acid (DADTPA, 134 mg, 0.38 mmol) is added to this solution and the mixture stirred overnight at room temperature. The solvent is removed *in vacuo* and the residue recrystallized from dichloromethane / ethyl ether to give 40.

25

A mixture of 40 (1.0 g, 0.025 mmol), 30 (170 mg, 0.20 mmol), and DMAP (76 mg, 0.6 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (20 mL) is stirred at room temperature for

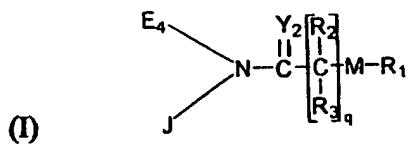
16 hours. The mixture is concentrated *in vacuo* and the residue is recrystallized from 2-propanol to give **41**.

5           The various publications, patents, patent applications and published applications mentioned in this application are hereby incorporated by reference herein.

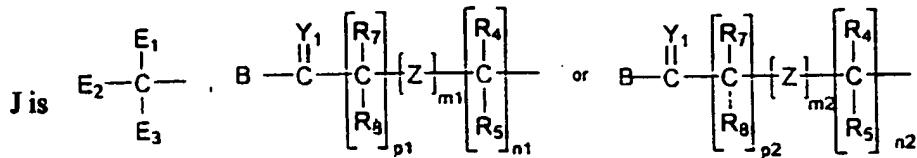
10           While there have been described what are presently believed to be the preferred embodiments of the invention, those skilled in the art will realize that changes and modifications may be made without departing from the spirit of the invention. It is intended to claim all such changes and modifications as fall within the true scope of the invention.

**WE CLAIM:**

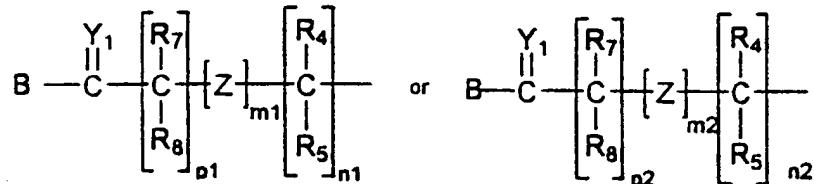
1. A compound comprising the formula:



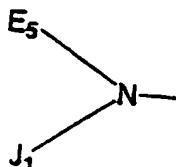
wherein:



$E_{1-4}$  are independently selected from the group consisting of hydrogen,  $C_{1-6}$  alkyls,  $C_{3-12}$  branched alkyls,  $C_{3-8}$  cycloalkyls,  $C_{1-6}$  substituted alkyls,  $C_{3-8}$  substituted cycloalkyls, aryls, substituted aryls, aralkyls,  $C_{1-6}$  heteroalkyls, substituted  $C_{1-6}$  heteroalkyls,  $C_{1-6}$  alkoxy, phenoxy,  $C_{1-6}$  heteroalkoxy,



and at least one of  $E_{1-4}$  includes a B moiety, wherein B is a leaving group, OH, a residue of a hydroxyl-containing moiety, a residue of an amine-containing moiety or



wherein  $J_1$  is the same as J, or another member of the group defining J and  $E_5$  is the same as  $E_{1-4}$ , or another member of the group defining  $E_{1-4}$ ;

$Y_{1-2}$  are independently O, S or  $NR_9$ ;

M is a heteroatom selected from either X or Q; wherein X is an electron withdrawing group and Q is a moiety containing a free electron pair positioned three to six atoms from C(=Y<sub>2</sub>);

R<sub>2-5</sub> and R<sub>7-9</sub> are independently selected from the group consisting of hydrogen, C<sub>1-6</sub> alkyls, C<sub>3-12</sub> branched alkyls, C<sub>3-8</sub> cycloalkyls, C<sub>1-6</sub> substituted alkyls, C<sub>3-8</sub> substituted cycloalkyls, aryls, substituted aryls, aralkyls, C<sub>1-6</sub> heteroalkyls, substituted C<sub>1-6</sub> heteroalkyls, C<sub>1-6</sub> alkoxy, phenoxy and C<sub>1-6</sub> heteroalkoxy;

(m1) and (m2) are independently zero or one;

(n1), (n2), (p1), (p2) and (q) are independently zero or a positive integer;

Z is an electron withdrawing group; and

R<sub>1</sub> is a polymeric residue.

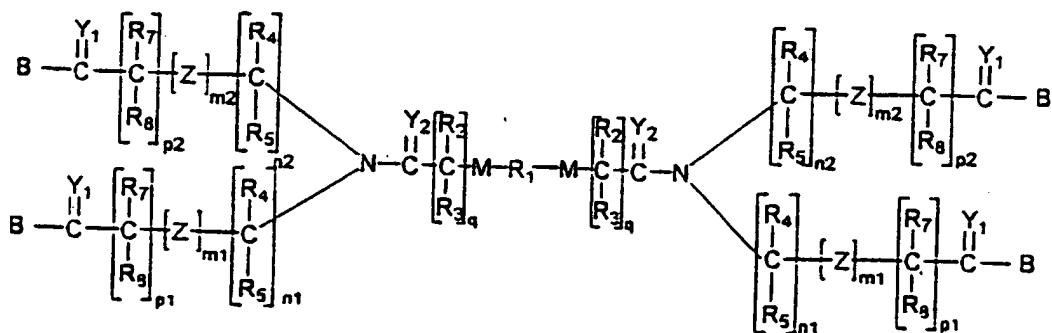
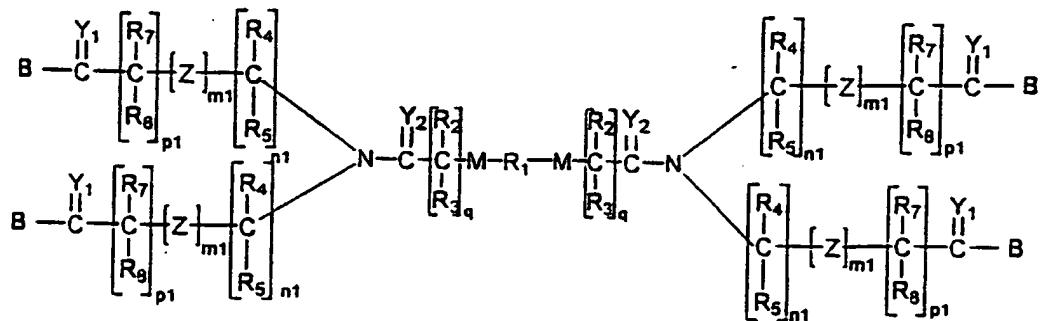
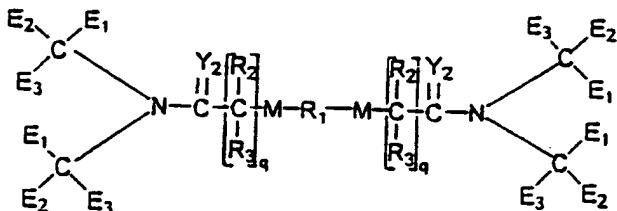
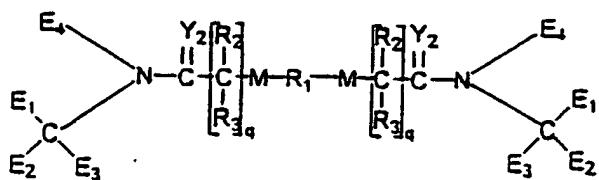
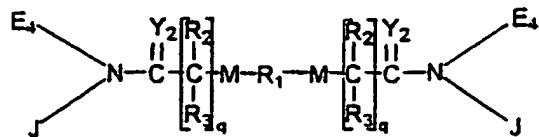
2. The compound of claim 1, wherein R<sub>1</sub> further comprises a capping group A, selected from the group consisting of hydrogen, CO<sub>2</sub>H, C<sub>1-6</sub> alkyl moieties, dialkyl acyl urea alkyls and

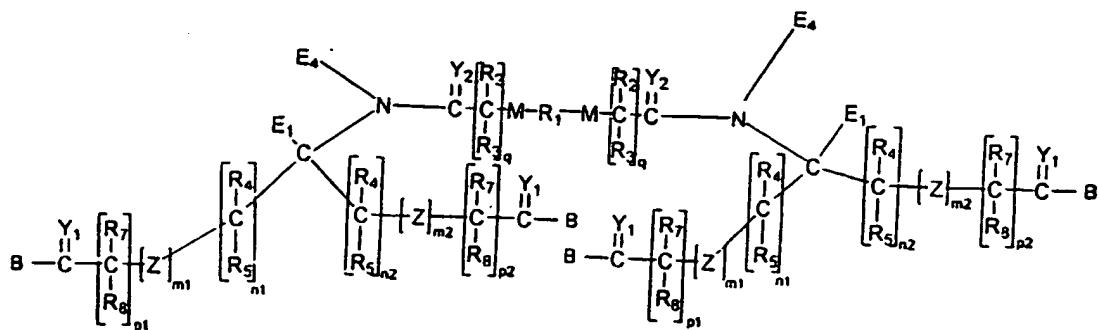


3. The compound of claim 1, wherein Y<sub>1</sub> and Y<sub>2</sub> are oxygen.
4. The compound of claim 1, wherein R<sub>2-5</sub>, R<sub>7</sub> and R<sub>8</sub> are hydrogen.
5. The compound of claim 1, wherein X is selected from the group consisting of O, S, SO, SO<sub>2</sub>, C(Y<sub>3</sub>) and NR<sub>6</sub>, wherein Y<sub>3</sub> is selected from the group consisting of O, S and R<sub>9</sub>, and R<sub>6</sub> is selected from the group consisting of hydrogen, C<sub>1-6</sub> alkyls, C<sub>3-12</sub> branched alkyls, C<sub>3-8</sub> cycloalkyls, C<sub>1-6</sub> substituted alkyls, C<sub>3-8</sub> substituted cycloalkyls, aryls, substituted aryls, aralkyls, C<sub>1-6</sub> heteroalkyls and substituted C<sub>1-6</sub> heteroalkyls.
6. The compound of claim 1, wherein Q is selected from the group consisting of C<sub>2-4</sub> alkyls, cycloalkyls, aryls, aralkyl groups substituted with a member of the group consisting of NH, O, S, -CH<sub>2</sub>-C(O)-NH-, and ortho-substituted phenyls.
7. The compound of claim 1, wherein (p1) and (p2) are 1.

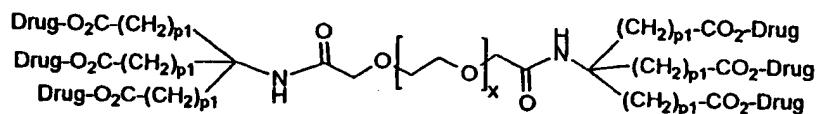
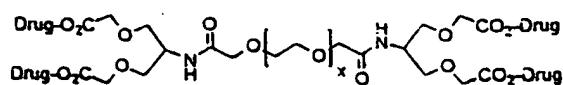
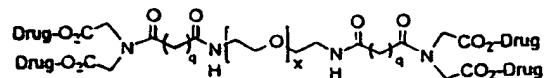
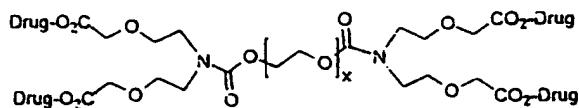
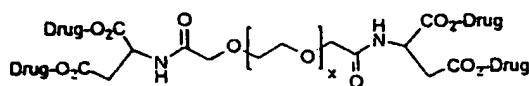
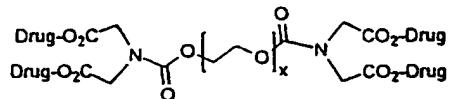
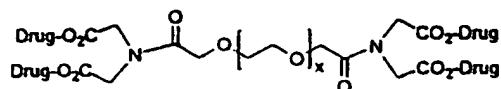
8. The compound of claim 1, wherein (n1) and (n2) are individually 1 or 2.
9. The compound of claim 8, wherein (n1) is 1 and (n2) is 2.
10. The compound of claim 1, wherein (m1) and (m2) are 1.
11. The compound of claim 1, wherein (q) is 1.
12. The compound of claim 1, wherein R<sub>1</sub> comprises a polyalkylene oxide residue.
13. The compound of claim 12, wherein said polyalkylene oxide residue comprises polyethylene glycol.
14. The compound of claim 1 wherein said polymeric residue has a molecular weight of from about 2,000 to about 100,000.
15. The compound of claim 14, wherein said polymeric residue has a molecular weight of from about 20,000 to about 40,000.
16. The compound of claim 1 wherein B is a residue of a member of the group consisting of Ara-C, camptothecin, camptothecin analogs, paclitaxel, taxoteres, gemcitabine, podophyllotoxin, fluconazole, ciclopirox, amphotericin B, nystatin, doxorubicin, daunorubicin, maytansine, vancomycin and erythromycin.
17. The compound of claim 1 wherein B is a residue of a member of the group consisting of anti-tumor agents; cardiovascular agents, anti-neoplastics anti-infectives, anti-fungals, anti-anxiety agents, gastrointestinal agents, central nervous system-activating agents, analgesics, fertility or contraceptive agents, anti-inflammatory agents, steroidal agents, anti-urecemic agents, cardiovascular agents, vasodilating agents and vasoconstricting agents.

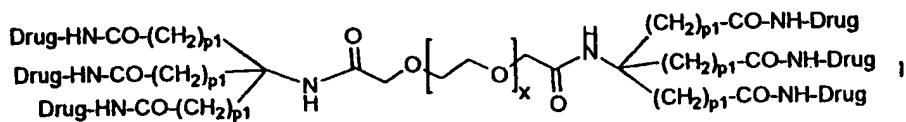
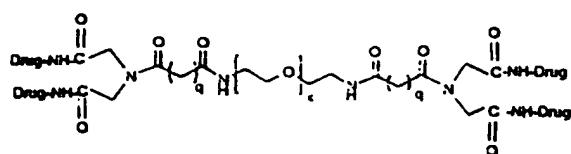
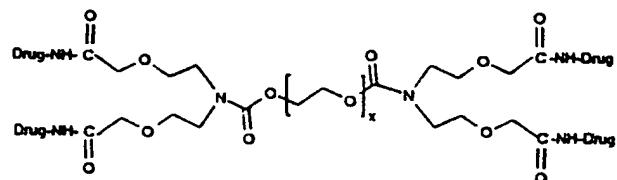
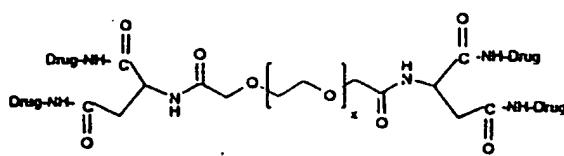
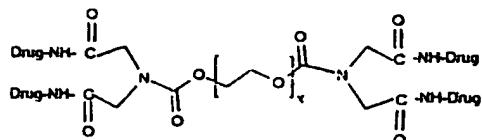
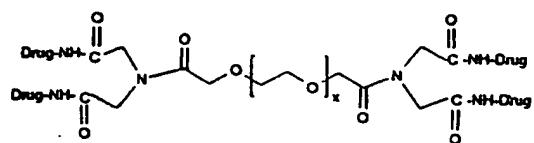
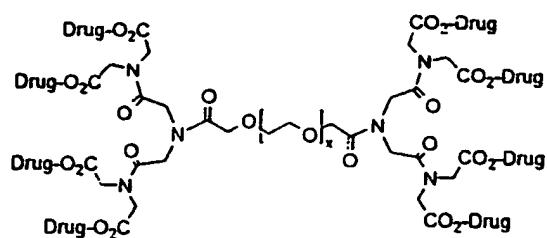
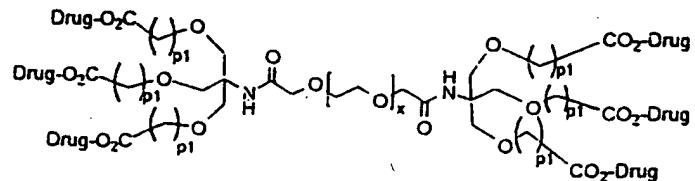
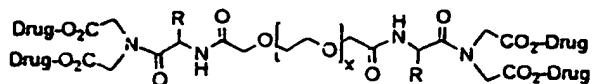
18. A compound of claim 1, having a formula selected from the group consisting of:

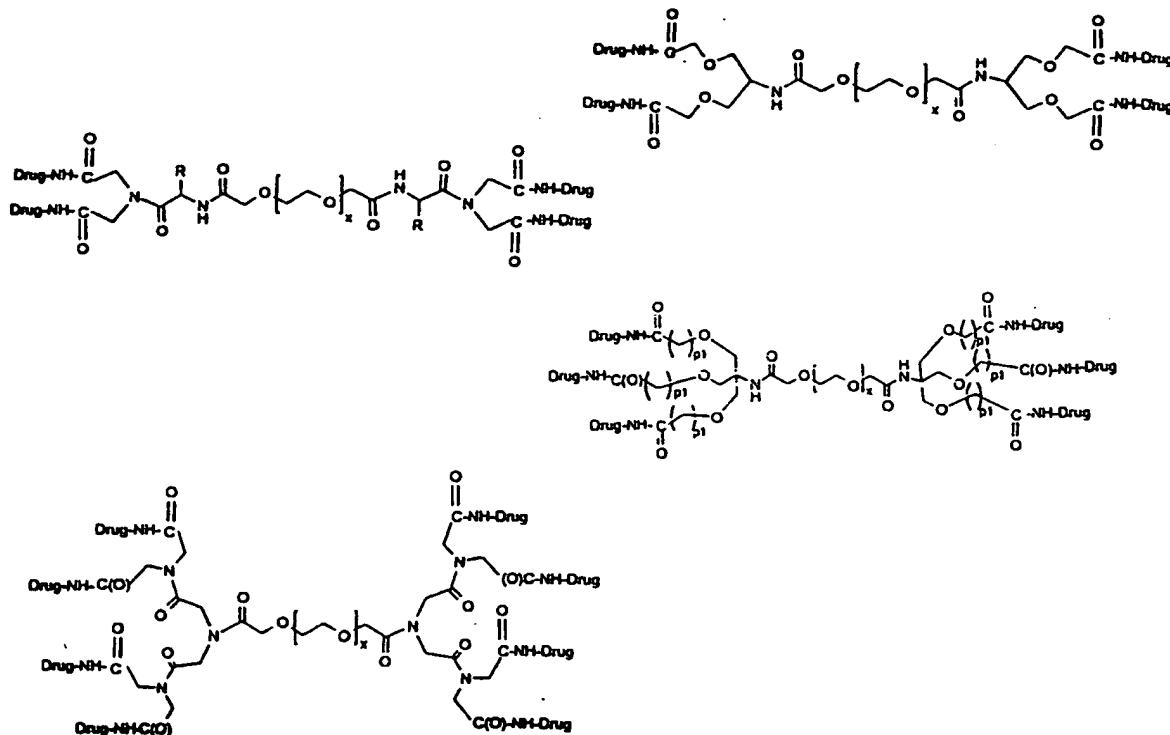




19. A compound of claim 1, having a formula selected from the group consisting of:



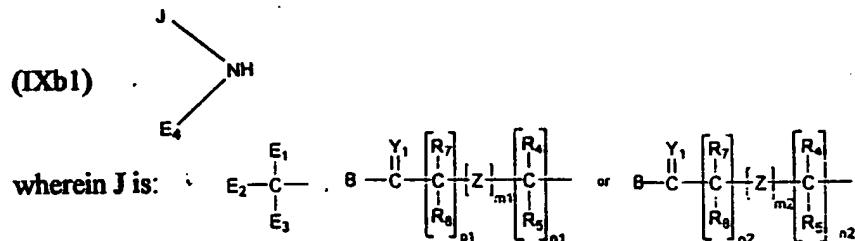




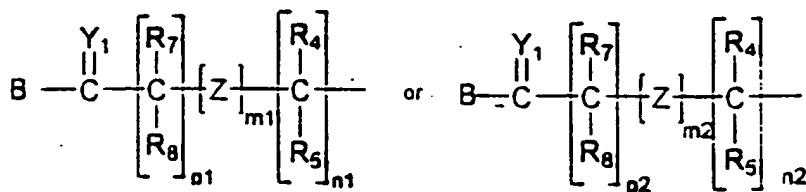
wherein (x) represents the degree of polymerization and "Drug" represents a residue of a hydroxyl- or amine-containing biologically active compound which has undergone a substitution reaction which results in the attachment of the biologically active moiety to the branched polymer.

**20. A method of preparing a polymeric transport system, comprising**

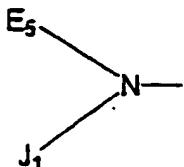
a) reacting a compound of formula:



$E_{1-4}$  are independently selected from the group consisting of hydrogen,  $C_{1-6}$  alkyls,  $C_{3-12}$  branched alkyls,  $C_{3-8}$  cycloalkyls,  $C_{1-6}$  substituted alkyls,  $C_{3-8}$  substituted cycloalkyls, aryls, substituted aryls, aralkyls,  $C_{1-6}$  heteroalkyls, substituted  $C_{1-6}$  heteroalkyls, substituted  $C_{1-6}$  heteroalkyls,  $C_{1-6}$  alkoxy, phenoxy,  $C_{1-6}$  heteroalkoxy,



and at least one of  $E_{1-4}$  includes a B moiety, wherein B is a leaving group, OH or



wherein  $J_1$  is the same as J, or another member of the group defining J and  $E_5$  is the same as  $E_{1-4}$  or another member of the group defining  $E_{1-4}$ ;

$Y_1$  is O, S, or  $NR_9$ ;

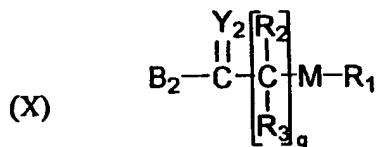
$R_{4-5}$  and  $R_{7-9}$  are independently selected from the group consisting of hydrogen,  $C_{1-6}$  alkyls,  $C_{3-12}$  branched alkyls,  $C_{3-8}$  cycloalkyls,  $C_{1-6}$  substituted alkyls,  $C_{3-8}$  substituted cycloalkyls, aryls, substituted aryls, aralkyls,  $C_{1-6}$  heteroalkyls, substituted  $C_{1-6}$  heteroalkyls, substituted  $C_{1-6}$  heteroalkyls,  $C_{1-6}$  alkoxy, phenoxy, and  $C_{1-6}$  heteroalkoxy;

(m1) and (m2) are independently zero or one;

(n1), (n2), (p1), and (p2) are independently zero or a positive integer; and

(Z) is an electron withdrawing group;

with a compound of the formula:



wherein

$B_2$  is a leaving group which is capable of reacting with an unprotected amine;

$Y_2$  is O, S, or  $NR_9$ ;

q is independently zero or a positive integer;

$R_{2,3}$  are selected from the group consisting of hydrogen,  $C_{1-6}$  alkyls,  $C_{3-12}$  branched alkyls,  $C_{3-8}$  cycloalkyls,  $C_{1-6}$  substituted alkyls,  $C_{3-8}$  substituted cycloalkyls, aryls, substituted aryls, aralkyls,  $C_{1-6}$  heteroalkyls, substituted  $C_{1-6}$  heteroalkyls, substituted  $C_{1-6}$  heteroalkyls,  $C_{1-6}$  alkoxy, phenoxy, and  $C_{1-6}$  heteroalkoxy;

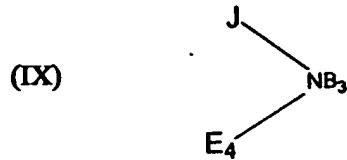
M is a heteroatom selected from either X or Q; wherein X is an electron withdrawing group and Q is a moiety containing a free electron pair positioned three to six atoms from  $C(=Y_2)$ ; and

$(R_1)$  is a polymeric residue; and

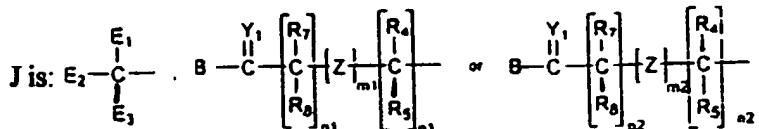
b) reacting the resultant compound with a sufficient amount of a biologically active moiety having a substitutable hydroxyl or amino group.

21. A method of preparing a polymeric transport system, comprising

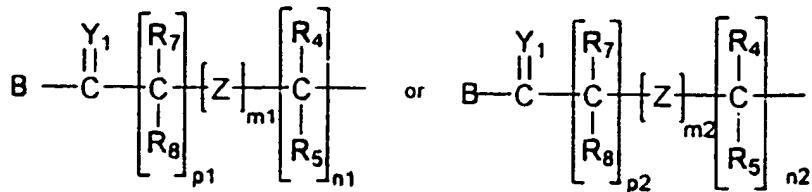
a) reacting a sufficient amount of a biologically active moiety having a substitutable hydroxyl or amino group with a compound of the formula:



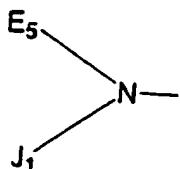
wherein  $B_3$  is a cleavable protecting group;



$E_{1,4}$  are independently selected from the group consisting of hydrogen,  $C_{1-6}$  alkyls,  $C_{3-12}$  branched alkyls,  $C_{3-8}$  cycloalkyls,  $C_{1-6}$  substituted alkyls,  $C_{3-8}$  substituted cycloalkyls, aryls, substituted aryls, aralkyls,  $C_{1-6}$  heteroalkyls, substituted  $C_{1-6}$  heteroalkyls, substituted  $C_{1-6}$  heteroalkyls,  $C_{1-6}$  alkoxy, phenoxy,  $C_{1-6}$  heteroalkoxy,



and at least one of  $E_{1-4}$  includes a B moiety, wherein B is a leaving group, OH, or



wherein  $J_1$  is the same as J, or another member of the group defining J and  $E_5$  is the same as  $E_{1-4}$  or another member of the group defining  $E_{1-4}$ :

$Y_1$  is O, S, or NR<sub>9</sub>;

R<sub>4-5</sub> and R<sub>7-9</sub> are independently selected from the group consisting of hydrogen, C<sub>1-6</sub> alkyls, C<sub>3-12</sub> branched alkyls, C<sub>3-8</sub> cycloalkyls, C<sub>1-6</sub> substituted alkyls, C<sub>3-8</sub> substituted cycloalkyls, aryls, substituted aryls, aralkyls, C<sub>1-6</sub> heteroalkyls, substituted C<sub>1-6</sub> heteroalkyls; substituted C<sub>1-6</sub> heteroalkyls, C<sub>1-6</sub> alkoxy, phenoxy, and C<sub>1-6</sub> heteroalkoxy;

(m1) and (m2) are independently zero or one;

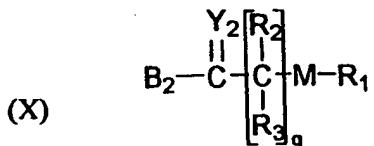
(n1), (n2), (p1), and (p2) are independently zero or a positive integer; and

(Z) is an electron withdrawing group;

with a biologically active moiety having a hydroxyl or amine group,

b) deprotecting the resultant intermediate by removing B<sub>3</sub>; and

c) reacting the deprotected intermediate compound with a compound of the formula



wherein

B<sub>2</sub> is a leaving group which is capable of reacting with an unprotected amine;

Y<sub>2</sub> is O, S, or NR<sub>3</sub>;

q is independently zero or a positive integer;

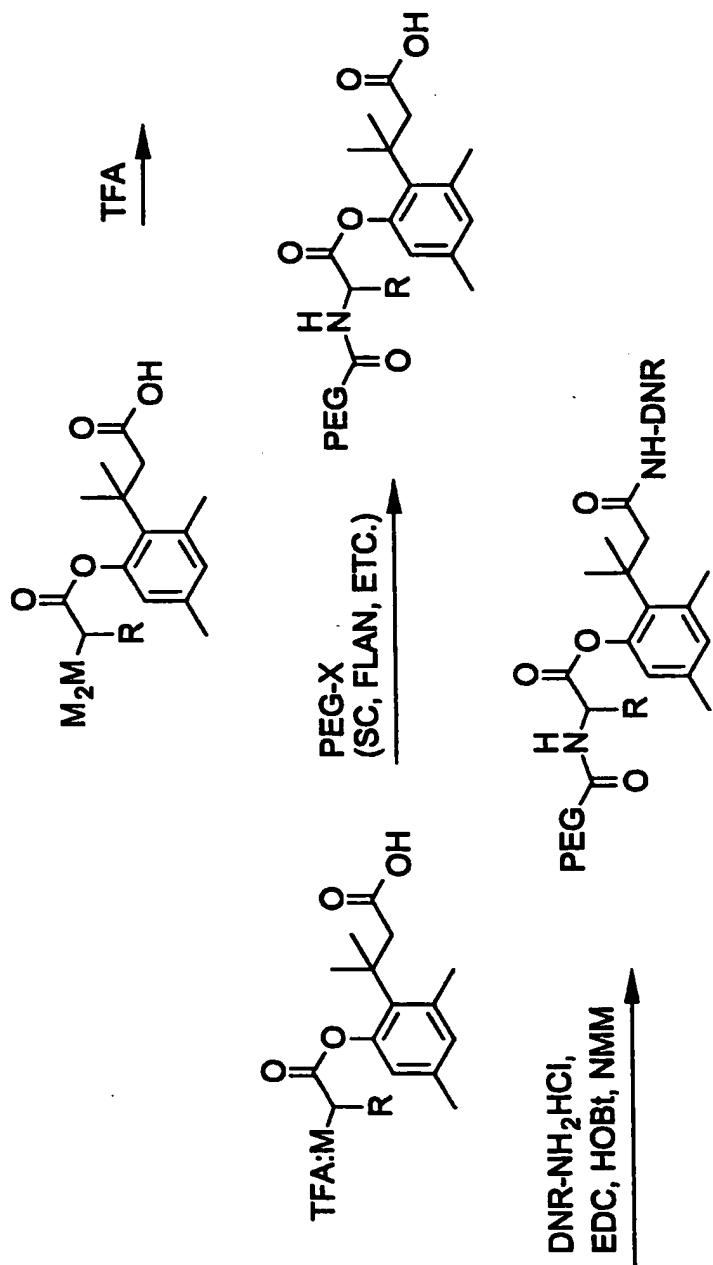
R<sub>2,3</sub> are selected from the group consisting of hydrogen, C<sub>1-6</sub> alkyls, C<sub>3-12</sub> branched alkyls, C<sub>3-8</sub> cycloalkyls, C<sub>1-6</sub> substituted alkyls, C<sub>3-8</sub> substituted cycloalkyls, aryls, substituted aryls, aralkyls, C<sub>1-6</sub> heteroalkyls, substituted C<sub>1-6</sub> heteroalkyls, substituted C<sub>1-6</sub> heteroalkyls, C<sub>1-6</sub> alkoxy, phenoxy, and C<sub>1-6</sub> heteroalkoxy;

M is a heteroatom selected from either X or Q; wherein X is an electron withdrawing group and Q is a moiety containing a free electron pair positioned three to six atoms from C(=Y<sub>2</sub>); and

(R<sub>1</sub>) is a polymeric residue.

22. A method of treating a mammal with prodrugs, comprising: administering to a mammal in need of such treatment an effective amount of a composition of claim 1 wherein B is a residue of a biologically active moiety.

FIG-1



2/11

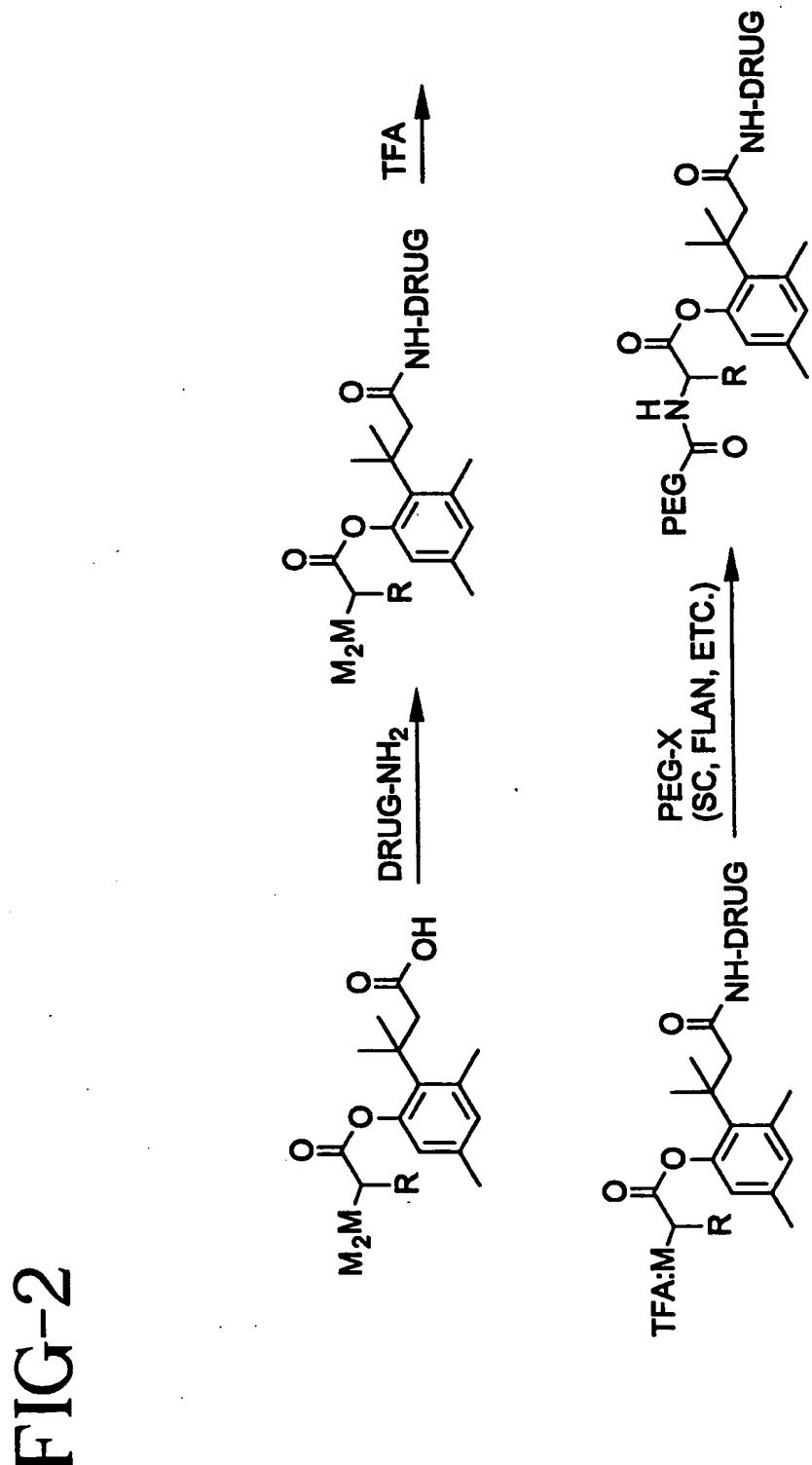
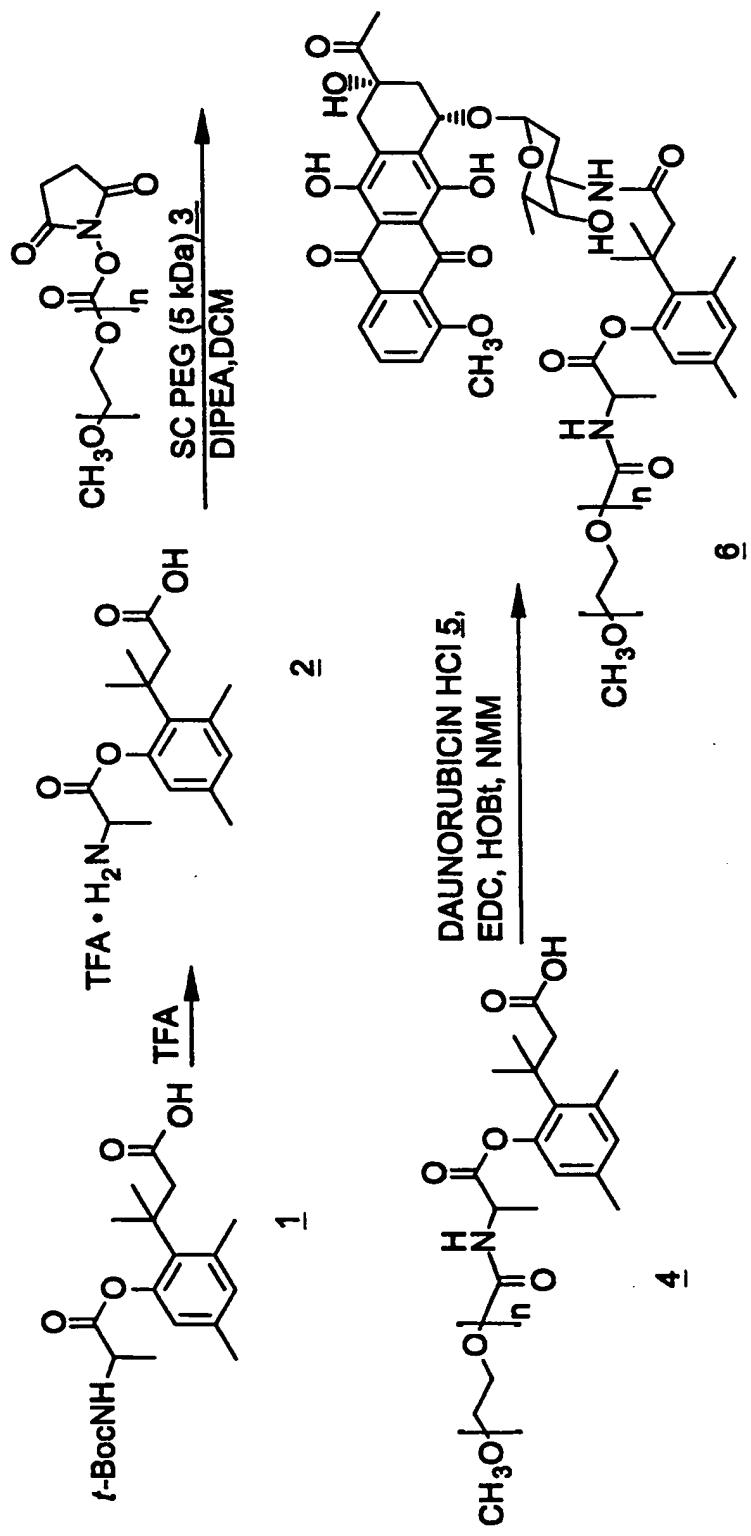


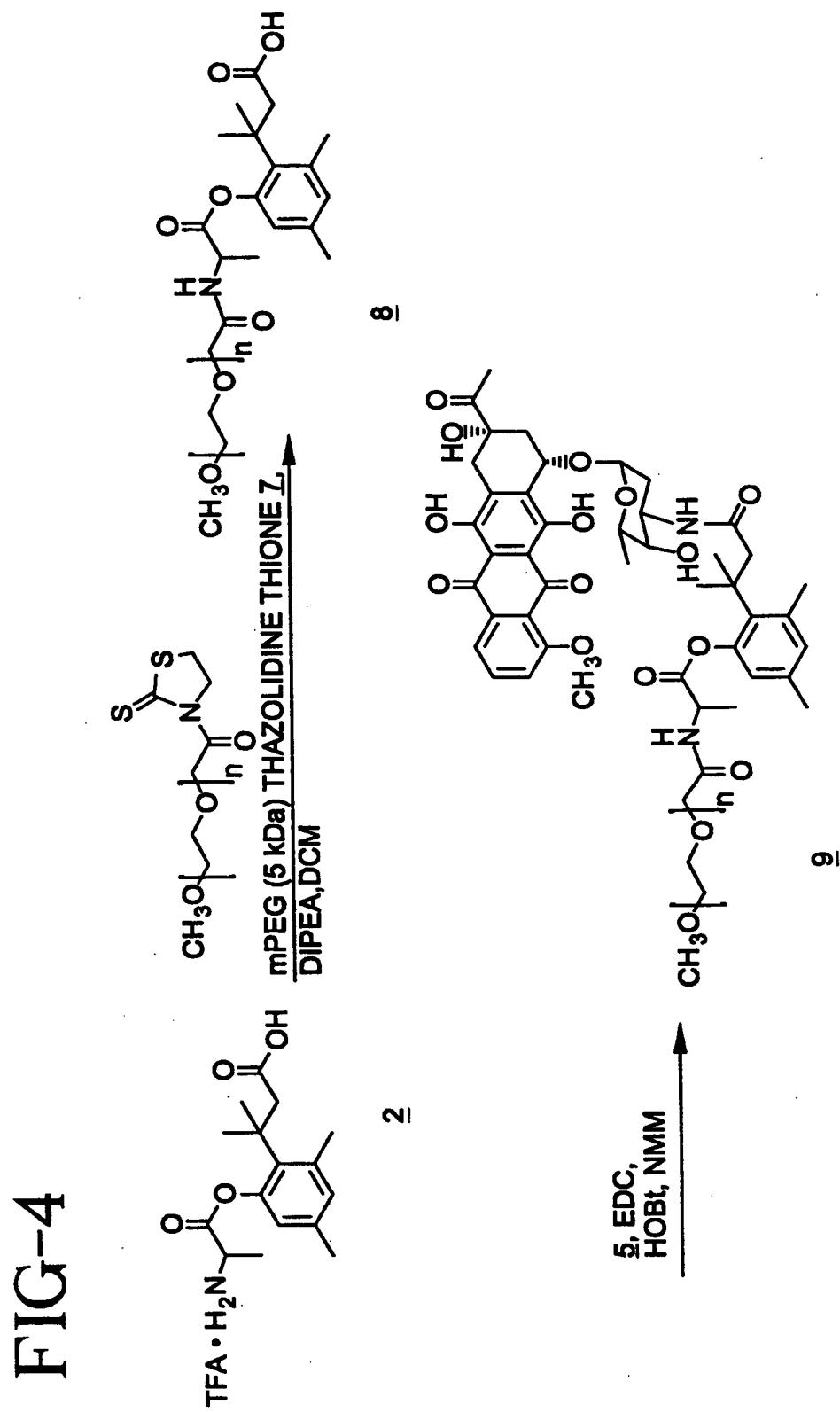
FIG-2

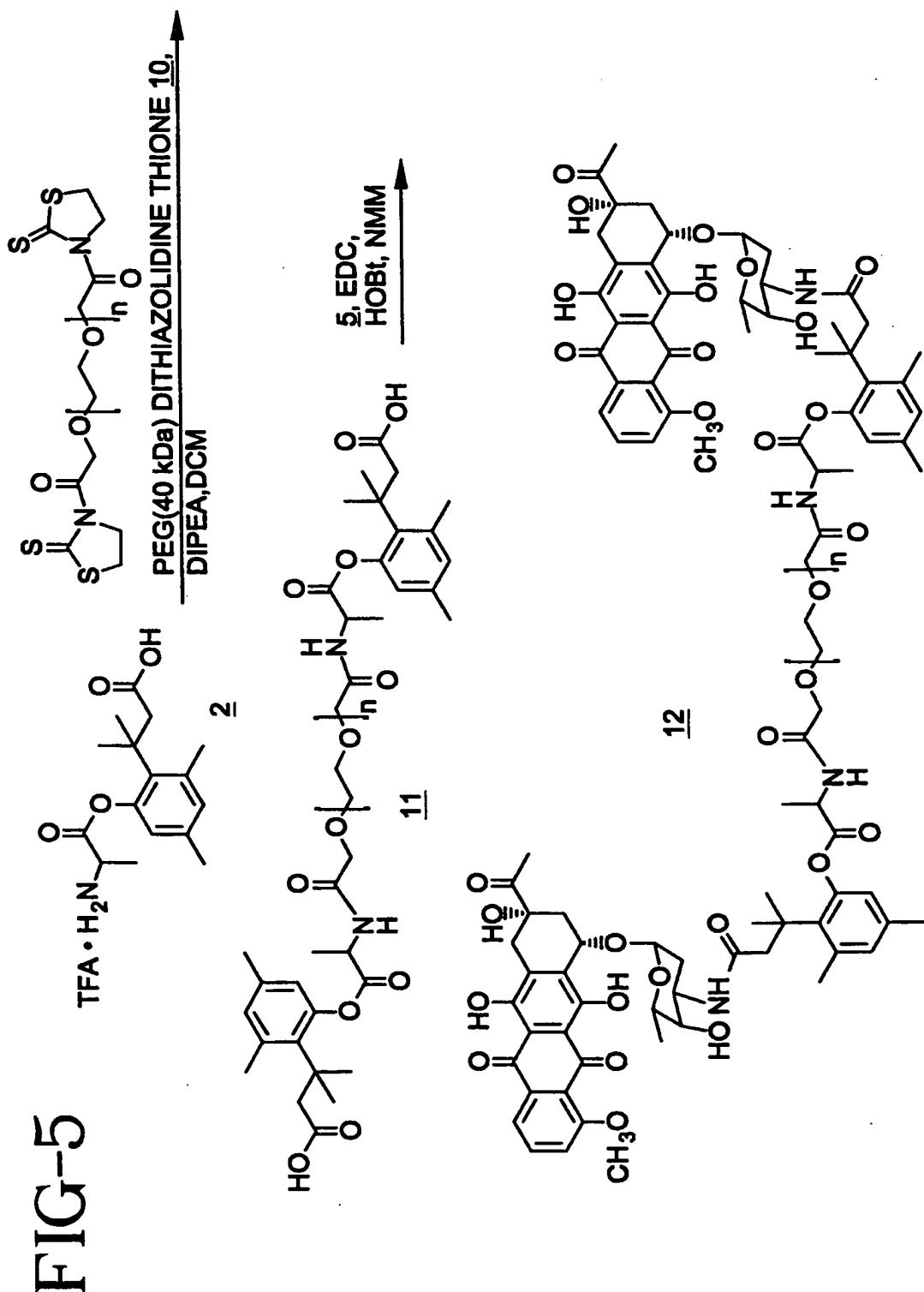
3/11

FIG-3



4/11





6/11

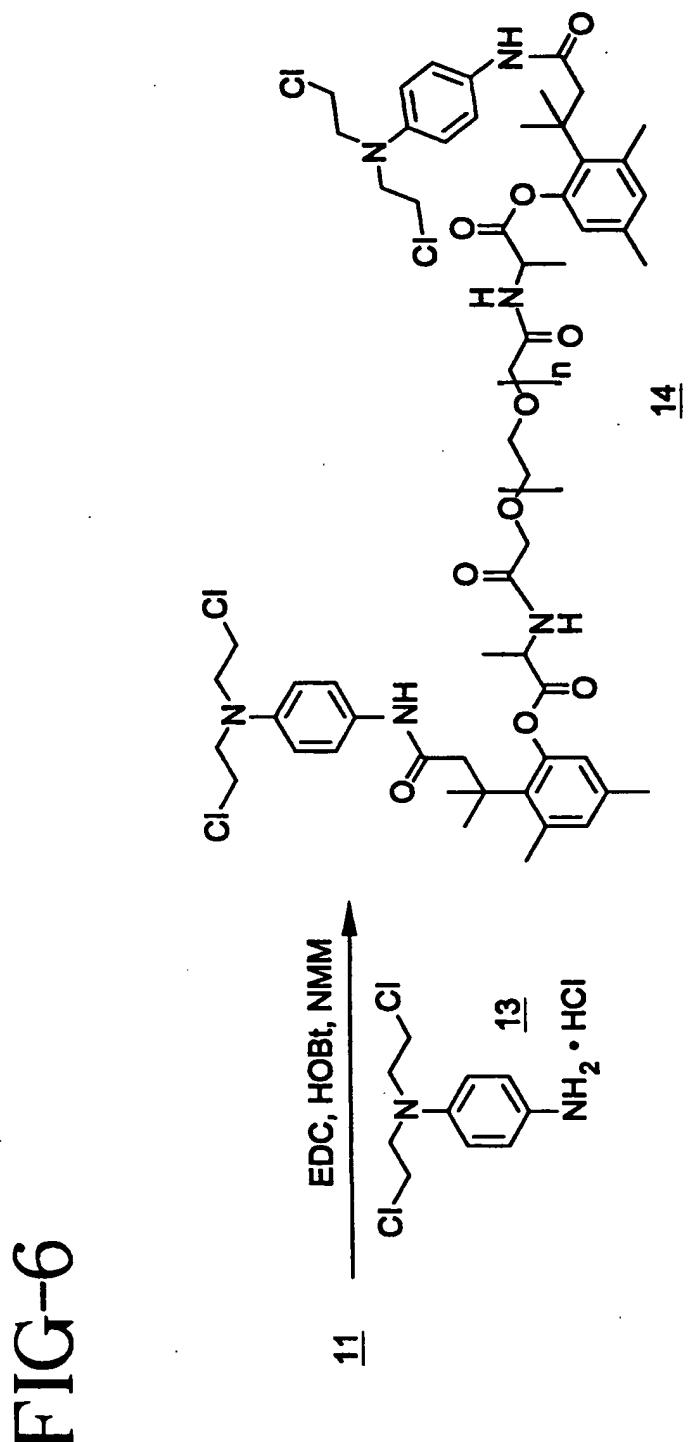


FIG-6

7/11

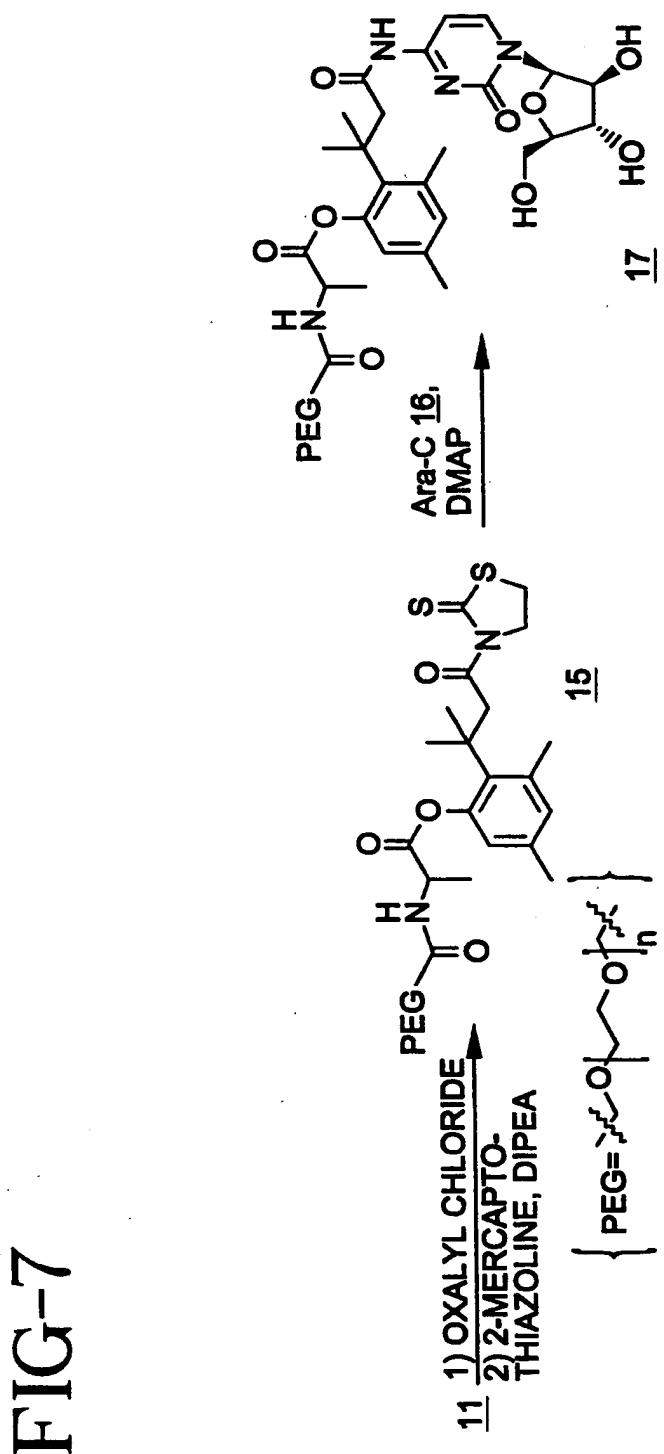
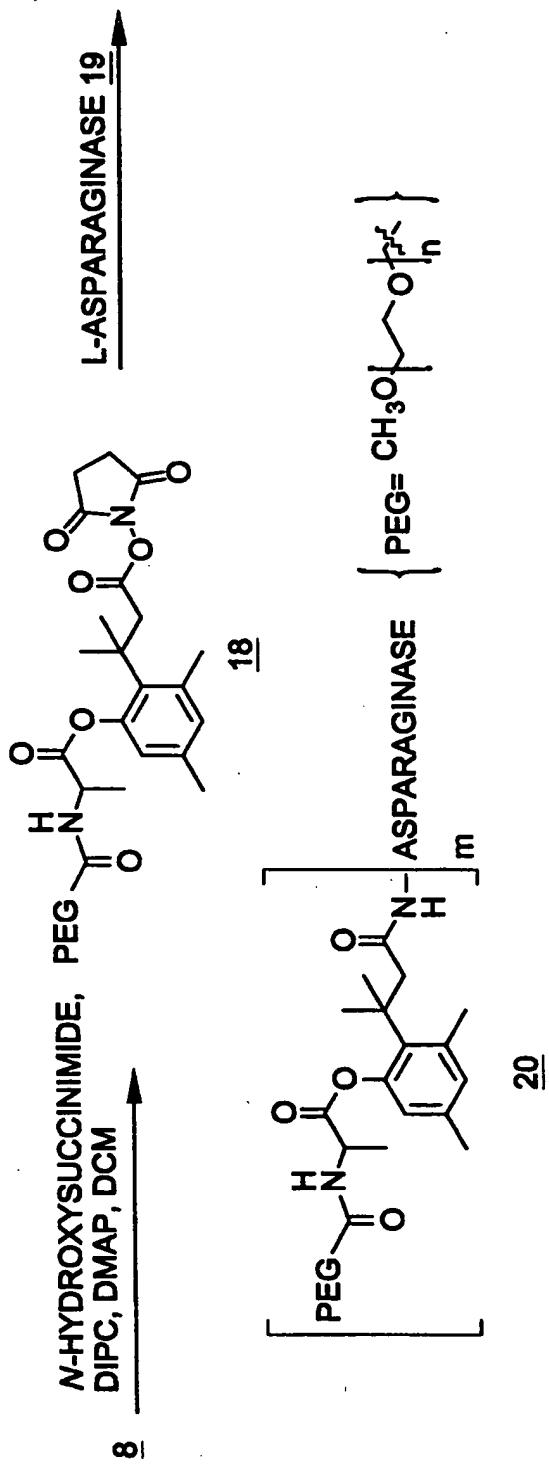
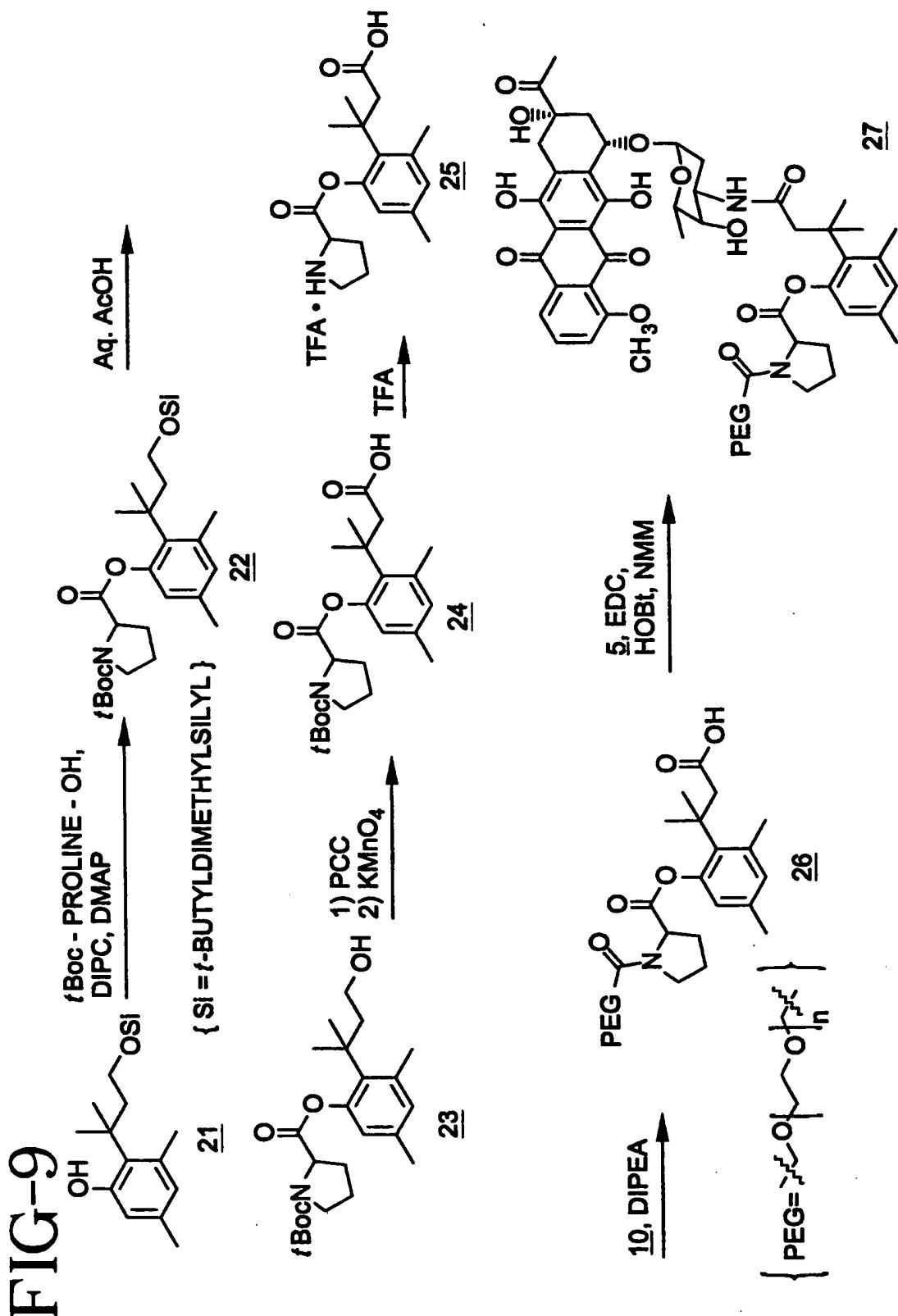


FIG-7

FIG-8

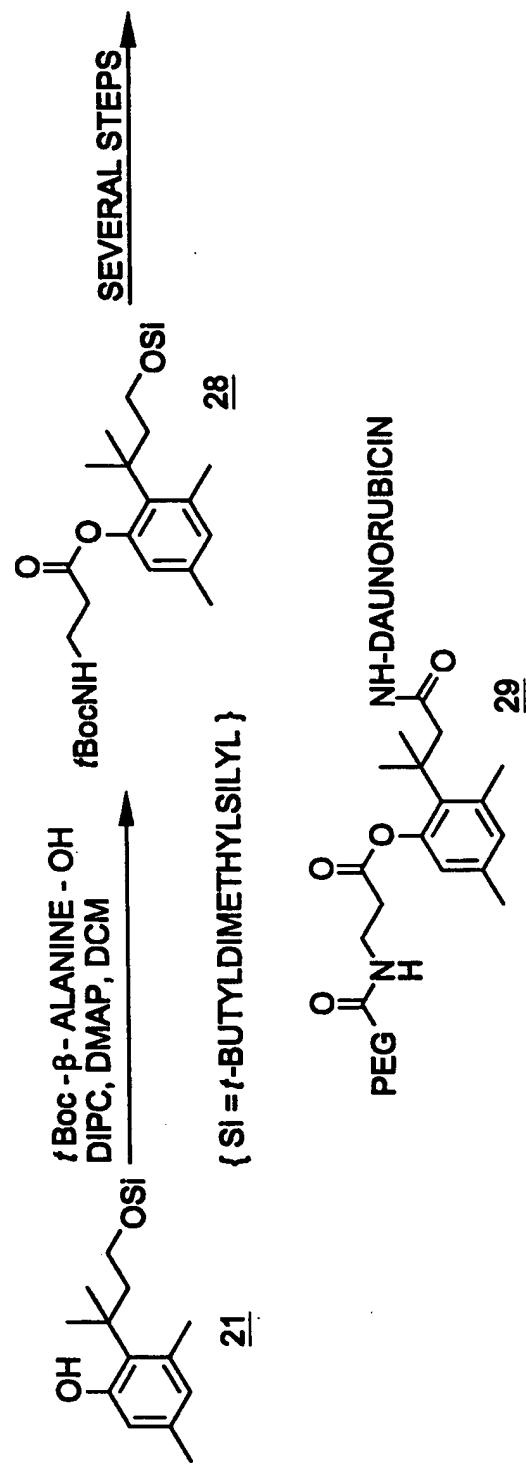


9/11



10/11

FIG-10



11/11

FIG-11



## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/08373

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) A61K 39/385; A01N 37/00, 43/02, 31/14; C08F 283/00

US CL :424/193.1; 514/529, 449, 715; 525/54.11

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/193.1; 514/529, 449, 715; 525/54.11

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN, MEDLINE, BIOSIS, REGISTRY, CAPLUS, SCISEARCH  
search terms: polymer-based prodrugs, branched conjugates, PEG

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,614,549 A (GREENWALD et al) 25 March 1997, see entire document.	1-22
Y	US 5,643,575 A (MARTINEZ et al) 01 July 1997, see entire document.	1-22
Y	OUCHI et al. Synthesis and Antitumor Activity of Poly(ethylene glycol)s Linked to 5-Fluorouracil Via a Urethane or Urea Bond. Drug Design Discov. 1992, Vol. 9, pages 93-105, see entire document.	1-22
Y	ZALIPSKY et al. Attachment of Drugs to Polyethylene Glycols. Eur. Polym. J. 1983, Vol. 19, No. 12, pages 117-1183, see entire document.	1-22

Further documents are listed in the continuation of Box C.  See patent family annex.

- \* Special categories of cited documents:
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "B" earlier document published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "Z" document member of the same patent family

Date of the actual completion of the international search

03 JULY 1999

Date of mailing of the international search report

20 AUG 1999

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized Officer

SUSAN HANLEY

Telephone No. (703) 308-0196

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/08373

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,547,981 A (GREENWALD et al) 20 August 1996, see entire document.	1-22
Y	US 5,622,986 A (GREENWALD et al) 22 April 1997, see entire document.	1-22
Y	US 5,693,310 A (GRIES et al) 02 December 1997, see entire document.	1-22